IMPROVED IDENTIFICATION OF HIGH RISK ENDOMETRIAL CARCINOMA

Yvette Geels

Publication of this thesis was generously sponsored by:
Olympus Nederland B.V.
Roche Nederland B.V.
ChipSoft B.V.
Bayer HealthCare Pharmaceuticals
Medical Dynamics
IKS International

Cover design: Florus Groot
Layout and printing: Proefschriftmaken.nl || Uitgeverij BOXPress, ‘s-Hertogenbosch
ISBN: 978-90-8891-923-7

IMPROVED IDENTIFICATION OF HIGH RISK ENDOMETRIAL CARCINOMA

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. mr. S.C.J.J. Kortmann,
volgens besluit van het college van decanen
in het openbaar te verdedigen op vrijdag 19 september 2014
om 10.30 uur precies

door

Yvette Pernella Geels
geboren op 24 mei 1982
te Loon op Zand

PROMOTOR	Prof. dr. L.F.A.G. Massuger
COPROMOTOREN	Dr. J.M.A. Pijnenborg (Twee Steden Ziekenhuis, Tilburg)
	Dr. J. Bulten
	Dr. M.P.M.L. Snijders (CWZ, Nijmegen)
MANUSCRIPTCOMMISSIE	Prof. dr. J.H. A.M. Kaanders (voorzitter)
	Prof. dr. I.D. Nagtegaal
	Prof. dr. R.F.P.M. Kruitwagen (Maastricht UMC)
PARANIMFEN	Drs. H.D.P. Luijks
	Drs. J.E.W. van Dijk

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE OF THE THESIS

General introduction

Endometrial carcinoma in general

Endometrial carcinoma is the sixth most common cancer in females and the second most common gynecologic cancer worldwide after carcinoma of the cervix. An estimated number of 287,100 new cases are diagnosed each year. In the western world, it is the most common gynecologic cancer.[1] In the Netherlands, it accounts for over 1900 newly diagnosed patients each year, and approximately 400 patients die as a consequence of the disease.[2] Two of the most important prognostic factors are tumor grade, and stage of the disease according to the 2009 FIGO staging system (International Federation of Gynecology and Obstetrics) (Table 1).[3,4] In general, endometrial carcinoma patients have a favorable prognosis, with a five-year overall survival rate of 80%. This relatively good prognosis can be partly attributed to the early presentation of the disease with postmenopausal vaginal blood loss or irregular premenopausal vaginal bleeding.[5]

Table 1: FIGO 2009 Staging System.

FIGO stage	Grade	Characteristics
I		Carcinoma confined to uterus
A	G123	No myometrial invasion or less < 50%
B	G123	Myometrial invasion > 50%
II	G123	Invasion of the cervical stroma
III		Metastases in the pelvic cavity
A	G123	Involvement of adnexa
B	G123	Involvement of the vaginal wall
C1	G123	Involvement of pelvic lymph nodes
C2	G123	Involvement of para-aortic lymph nodes
IV		Distant metastases
A	G123	Abdominal metastases outside the pelvic cavity
B	G123	Thoracal metastases

G1: 5% or less of a nonsquamous or nonmorular solid growth pattern
G2: 6-50% of a nonsquamous or nonmorular solid growth pattern
G3: more than 50% of a nonsquamous or nonmorular solid growth pattern
Notable nuclear atypia inappropriate for the architectural grade, raises a grade 1 or grade 2 tumor by one grade
Clearcell adenocarcinoma and serous adenocarcinoma are grade 3 tumors

The appearance of normal endometrium

The endometrium in premenopausal women shows a cyclic pattern of proliferative, secretory, and declining endometrium.[6] The endometrial lining in postmenopausal women is expected to be atrophic.[7-10] However, only few papers report on the appearance of "normal" postmenopausal endometrium. In addition, proliferative endometrium to some extent has been reported in a significant part of asymptomatic postmenopausal women.[11] In order to understand the carcinogenesis of endometrial carcinoma, it seems necessary to know the normal appearance of both pre- and postmenopausal endometrium, and to determine the nature of the background endometrium found in the histologic specimens of uteri with endometrial carcinoma.

Histopathology and carcinogenesis

In endometrial carcinoma several histopathologic types are distinguished: endometrioid adenocarcinoma, mucinous adenocarcinoma, serous adenocarcinoma, clear cell adenocarcinoma, undifferentiated carcinoma, and mixed carcinoma (composed of more than one type, with at least 10% of each component).[5] These histopathologic classifications are grouped in two different subtypes. This widely accepted dualistic model of endometrial carcinogenesis was proposed by Bokhman in 1985.[12] He based the subdivision of endometrial carcinoma in two different types on observations of clinical behavior and histopathologic presentation of the carcinoma.

Type I carcinoma, representing 80% of all endometrial carcinomas, occurs around an average age of 60 years and bears in general a good prognosis. These carcinomas are linked to unopposed estrogen stimulation caused by obesity, nulliparity, and exogenous hormone use. Unopposed estrogen stimulation may result in the development of simple hyperplasia of the endometrium, which may progress to atypical hyperplasia, and ultimately results in endometrioid endometrial carcinoma (EEC). Type I carcinomas are characterized by ER (estrogen) and PR (progesterone) expression, Micro Satellite Instability, and alterations in *K-Ras, CTNNB1, PTEN,* and the WNT-pathway.[13-19]

Type II carcinomas, on the contrary, occur at an average age of 70 years, and bear a relatively poor prognosis. These carcinomas show non-endometrioid histology like serous and clear cell type, and are not linked to estrogen stimulation. The background endometrium of these

patients appears atrophic, and carcinomas are characterized by aneuploidy, p53 and Her2/neu expression, and loss of E-cadherin expression.[13-19] Table 2 shows an overview of the clinical and pathologic features in type I and type II carcinomas.

However, about 20% of the individual cases does not fit into this dualistic model, represented by endometrioid endometrial carcinomas with poor prognosis.[14,20,21] Is has been suggested that a third type endometrial carcinoma exists: EEC with atrophic background endometrium and poor prognosis.[21]

Table 2: Clinical and pathologic characteristics in type I and type II endometrial carcinomas.

Type I	Type II
60 years	70 years
Estrogen stimulated	Not estrogen stimulated
Endometrioid type	Non-endometrioid type
Good prognosis	Poor prognosis
Hyperplasia of background endometrium	Atrophy of background endometrium

Surgical treatment

The cornerstone of endometrial carcinoma treatment is surgery. The standard treatment for clinical FIGO stage I disease includes hysterectomy and bilateral salpingo-oophorectomy. The further extent of the surgical treatment, and mainly the extent of lymphadenectomy has been a matter of debate for decades. In two large randomized multicentre trials, no survival benefit of routine lymphadenectomy could be demonstrated in apparently FIGO stage I endometrial carcinoma patients.[22,23] However, many critical comments on the methodology of these trials have been expressed, and the last word has not been said in this worldwide controversy.[24]

In the Netherlands, the guidelines of endometrial carcinoma treatment have recently been updated.[2] For endometrioid endometrial carcinomas, with tumor grade 1 or 2, only hysterectomy with bilateral salpingo-oophorectomy is recommended. For tumor grade 3 endometrioid carcinomas complete surgical staging is to be considered. For serous and clear cell carcinomas a complete surgical staging procedure is recommended. Complete staging

includes total abdominal hysterectomy, bilateral salpingo-oophorectomy, pelvic washings, bilateral pelvic and para-aortic lymph node sampling and omentectomy or omental biopsy.[25]

Adjuvant treatment

The most important adjuvant therapeutic strategy in endometrial cancer treatment is radiotherapy, for which in the Netherlands, the PORTEC criteria are leading.[26,27] The PORTEC I trial revealed that FIGO stage I patients, with two out of three risk factors (age above sixty years, tumor grade 3 and myometrial invasion more than 50%) do benefit from external beam radiotherapy for loco-regional control of disease.[26] In addition, the PORTEC II trial revealed that vaginal brachytherapy is equally effective in preventing loco-regional recurrence when compared to external beam radiotherapy, with fewer gastrointestinal toxic effects.[27] In patients with stage III or IV disease, or serous or clear cell histologic type, (neo) adjuvant platinum based chemotherapy can be considered.[28]

Individualized treatment

Individualization of treatment is more and more important in this carcinoma with a relatively favorable prognosis, but with individual cases that present with an aggressive clinical course. It is challenging to identify those patients that need a more aggressive treatment, without over-treating the patients who are sufficiently treated with standard management. This way, recurrence of disease due to under-treatment, and morbidity and side-effects caused by unnecessary treatment can be prevented. To make accurate treatment decisions it is of great importance to be able to predict the clinical behavior of a carcinoma. Prediction of clinical behavior can be guided by histopathologic and immunohistochemical or molecular markers. These markers can be helpful both in preoperative and postoperative decision making.

Clinicopathologic markers

The decision of the extent of surgical therapy is primarily based on the histopathologic characteristics of the pre-operative biopsy of the endometrium. Biopsies can be obtained in the outpatient department using pipelle, or in a clinical setting by Dilatation & Curettage or hysteroscopy.[29] It is obligatory to determine tumor type and tumor grade of the endometrial carcinoma in the biopsy. However, making the correct diagnosis based on

often small biopsies can be challenging. A second risk of misclassification is the possible heterogeneity of the tumors, when only a part of the tumor is represented in the biopsy. The postoperative reproducibility of the tumor grade based on preoperative endometrial biopsies obtained with pipelle, Dilatation & Curettage, or by hysteroscopy varies from 59% to 96% in literature.[30-33] Therefore, for a substantial part of the patients the extent of surgical treatment may possibly not be chosen correctly.

A second tool, which can be of possible help in the pre-operative setting, is cervical cytology. It has been proposed by several authors that cervical cytology can give direction in the diagnostic work-up of endometrial carcinoma patients. Malignant endometrial cells found in cervical cytology are associated with parameters of poor prognosis like more advanced FIGO stage and high tumor grade.[34-36] Some authors suggest that positive cervical cytology is associated with serous or clear cell histologic type.[37,38]

In addition, surgical therapy is based on clinical estimation of the FIGO stage of disease. Risk estimation for the presence of extended disease is challenging as well. For instance, in FIGO stage II disease, for which more extended surgery with pelvic lymphadenectomy is recommended, the pre-operative determination of cervical involvement may be complex. MRI is the most accurate way of defining cervical invasion, but does not show an acceptable sensitivity.[39] Equally, MRI is the most accurate way of assessing myometrial invasion pre-operatively. However, due to its low negative predictive value, it is not a useful screening tool in determining the absence of myometrial invasion.[40] The identification of stage III or IV disease, i.e. metastases in lymph nodes or other distant metastatic sites, with imaging techniques is also prone to misinterpretation. CT and MRI are equivalent in terms of evaluating nodal metastases, but neither is sensitive enough to replace surgical lymph node assessment.[5] An algorithm which predicts the presence of lymph node metastases has been developed by examination of myometrial invasion, tumor grade, and tumor diameter intra-operative.[41] However, this setting during operation bears logistic challenges, and it may not be implemented widely.

Additionally, serum markers have been studied for their prognostic value in endometrial carcinoma. CA125 (cancer antigen 125), the most common used marker in endometrial cancer has been found to be elevated in 25% of the patients, providing information on potential extended disease outside the uterus.[42] A more promising serum marker may be HE4 (human epididymis protein 4). HE4 can be helpful in predicting myometrial invasion and

high tumor grade. However, a correlation with the presence of lymph node metastases has not been proven, yet.[43] Potentially, a combination of both tumor markers may better predict the presence of metastatic disease than either one alone.[44]

Finally, as mentioned before, in the postoperative setting myometrial invasion and tumor grade are crucial in determining adjuvant treatment strategy.[26] For tumor grading, the pre-operative diagnosis needs to be evaluated on the hysterectomy specimen. In case of doubt about the diagnosis, immunohistochemical markers can be of additional help, which will be discussed later in this chapter. The assessment of myometrial invasion bears a substantial deal of intra-observer variability. Irregular endomyometrial junctions, exophytic tumors, smooth muscle metaplasia, and adenomyosis make the assessment of myometrial invasion challenging.[45] In literature, other, possibly better, reproducible ways of assessing myometrial invasion have been proposed.[46-48]

Immunohistochemical markers

Many immunohistochemical markers and changes of molecular pathways are known to be predictive for clinical outcome in endometrial carcinoma patients. Some of these markers have been known for years already. Surprisingly, these markers are still not used to give some direction in correctly diagnosing and treating patients with suspicion of endometrial cancer, like in breast cancer for example. The best known markers in the prediction of disease free- and overall survival in endometrial carcinoma patients are the expression of p53, MIB1, and loss of ER and PR expression.[49-53] Other identified markers are changes in expression of β-catenine, a gain in expression of stathmin and PIK3CA, and loss of MLH1, E-cadherin, p21, and p16 expression.[14,15,20,51,54-58] In addition, expression of L1CAM has been identified to be a promising marker in endometrial carcinomas.[59-61] L1CAM has recently shown to be a strong predictive marker in a large cohort of early stage EEC patients.[62]

Most of these markers are also known to be helpful in the distinction between type I and type II carcinomas, again reflecting the prognostic information.[63] All markers associated with type II carcinomas are associated with poor prognosis as well. Table 3 shows an overview of the changes of several pathways and markers in type I and type II carcinomas. An overview of the best studied pathways in endometrial carcinoma is shown in Figure 1.[14]

Table 3: Immunohistochemical and genetic changes in type I and II endometrial carcinomas.

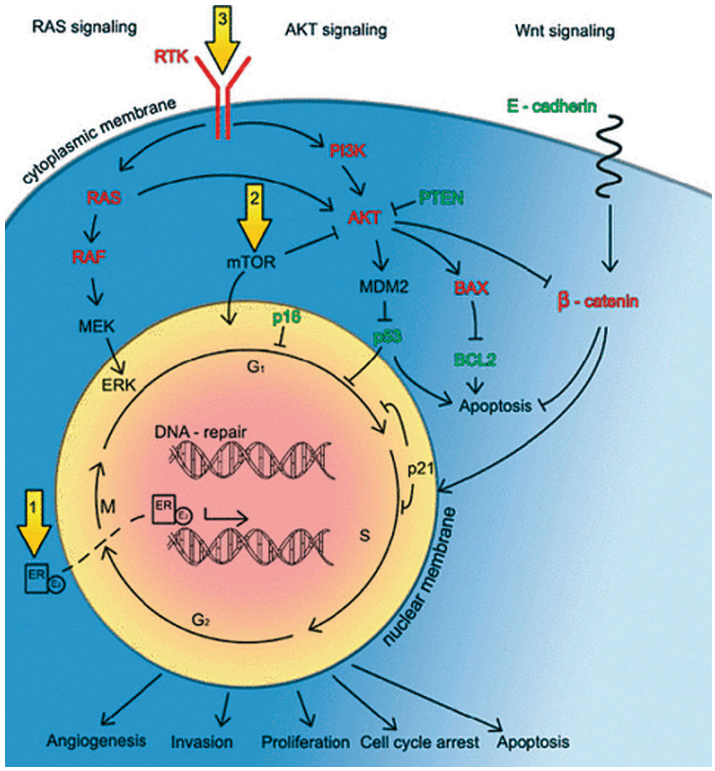
Marker	Function	Type I	Type II
Microsatellite instability	Defect mismatch repair genes, accumulation mutations microsatellites	20-40%	0-5%
K-RAS mutation	Ras-raf-MAP/ RAS signaling, oncogene, signal transduction, cell proliferation	13-40%	0-10%
B-RAF mutation	Ras-raf-MAP/ RAS signaling, mediates cell growth and malignant transformation	23%	11%
↓MLH1, MSH2	Mismatch repair genes	20-40%	0-10%
↓PTEN	PI3Kinase pathway, tumor suppressor gene, regulation cell cycle	30-60%	0-11%
↑PIK3CA	PI3Kinase pathway, oncogene, mutations co-existing with PTEN mutations	24-36%	24-36%
↑Stathmin	PI3Kinase pathway, oncoprotein, promotes proliferation	in aggressive EC*	
↑/↓β-catenine	WNT pathway, specification cell fate, regulation proliferation, dual function in cell signaling and adhesion	14-44%	0-5%
↑p53	Tumor suppressor gene, cell proliferation and apoptosis	5-20%	71-90%
↑/↓p21	Downstream effector in P53 pathway	in aggressive EC	
↑/↓p16	Tumor suppressor gene, cell cycle regulator	10%	10-40%
↑HER-2/*neu*	Oncogene, epidermal growth factor receptor, cell growth and differentiation	3-10%	18-80%
↑L1CAM	Transmembrane cell adhesion molecule	in aggressive EC	
↓E-cadherin	Transmembrane cell adhesion molecule	10-43%	57-90%
↓ER/PR [14]	Steroid receptor, transcription factors	70-73%	19-24%
↑MIB1 (Ki-67)	Proliferation marker	53%	62%
↑Insulin Like Growth Factor	Normal growth/development, mediating steroid hormone actions	in aggressive EC	
↑VEGF	Vascular endothelial growth factor	in aggressive EC	

*Endometrial Carcinoma

Mechanisms of metastasizing

To be able to predict which patient actually has or will develop metastatic disease, more insight in the mechanisms of metastasizing in endometrial carcinomas is needed. Endometrial carcinoma spreads by exfoliation of cells that are shed through the fallopian tubes, lymphatic dissemination and/or hematogenous dissemination. The metastatic

Figure 1: The RAS signaling, AKT signaling and Wnt signaling pathway in endometrial carcinoma.



From: Engelsen IB, Akslen LA, Salvesen HB. Biologic markers in endometrial cancer treatment. APMIS. 2009;117(10):693-707. Epub 2009/09/25.

process starts with the direct invasion of the tumor in the surrounding tissue: the myometrium. For this process to occur, epithelial tumor cells need to undergo an epithelial to mesenchymal transition (EMT).[64] In EMT, epithelial cells lose their polarity and cell-cell contacts, undergoing a dramatic remodeling of the cytoskeleton end acquiring a migratory phenotype.[65] This process has been extensively described in other types of cancer, and the cellular and molecular steps required for metastasis may be similar for all cancer cells.[66] Recently, few insights have been given in EMT in endometrial carcinoma. Some hallmarks of EMT have been reported in endometrial carcinoma either at the level of E-cadherin loss or the induction of its repressors.[64,67] In addition, other molecular alterations are correlated with carcinomas with expression of EMT hallmarks. For example, L1CAM expression is correlated with loss of E-cadherin expression.[61] After invasion of the myometrial tissue, and possibly the lymphatic or blood vessels, cancer cells need to arrive and grow in sites distant

from the primary tumor.[68] Also, for this step in the metastatic process few markers have been reported in endometrial carcinoma. For example, the expression of vascular endothelial growth factor (VEGF) and Insuline-like growth factor-1 receptor (IGF-1R) were associated with aggressive phenotype and lymph node metastases in endometrial carcinoma.[69,70] Taking all this together, although some markers in the metastatic process of endometrial carcinoma have been identified, essential parts of the mechanisms behind the metastatic process in endometrial carcinoma are still poorly understood.

Aims of the thesis

Decades of research on endometrial carcinoma have given us clinical and histopathologic tools to decide on the appropriate treatment for the individual patient. Nevertheless, the individual course of disease of a patient can (unexpectedly) be more aggressive than predicted based on these tools. Therefore, more research is needed in histopathologic and immunohistochemical characteristics of endometrial carcinoma for a more accurate guidance of decision making in the surgical and adjuvant treatment of these patients. In addition, to be able to investigate the pathologic condition of the endometrium, the appearance of the normal endometrium should be exactly known as a reference. The aims of this thesis were therefore:

- To accurately study and describe the endometrium in asymptomatic pre- and postmenopausal women.
- To accurately study and describe the endometrium adjacent to carcinoma.
- To investigate the prognostic value of pathologic markers.
- To gain more insight in the pathologic and immunohistochemical markers in patients not fitting in the dualistic model of Bokhman.
- To assess the prognostic value of immunohistochemical markers in endometrioid endometrial carcinoma.
- To gain more insight in the metastatic process of endometrioid endometrial carcinoma.

Outline of the thesis

In Chapter 2, a detailed description of the endometrium in pre- and postmenopausal women without symptoms of endometrial disease is given. To this end, the entire endometrium of patients who underwent a hysterectomy because of uterine prolapse was systematically sampled according to the SEE-END protocol.

In Chapter 3, the prevalence and clinical value of endometrial cells found in cervical smears of a cohort of endometrioid endometrial carcinoma patients and a cohort of uterine papillary serous carcinomas is investigated.

In Chapter 4, the prognostic value of the depth of myometrial invasion in absolute millimeters is evaluated and compared to the currently used cut-off value of invasion of more or less than 50% of the myometrium.

In Chapter 5, the background endometrium of endometrioid endometrial carcinomas is reviewed. The prognostic value of the background endometrium is evaluated. Since the findings of the study show a subgroup of patients not fitting in the dualistic model of type I and type II carcinomas the existence of a third type endometrial carcinoma is proposed.

In Chapter 6, the expression of a set immunohistochemical markers and the mutation analysis of three genes is performed in a group of type I, type II and the proposed type III endometrial carcinoma, to find evidence to support the theory of the existence of a third type endometrial carcinoma.

In Chapter 7, an immunohistochemical comparison is made between endometrioid endometrial carcinomas with and without metastases to be able to identify markers associated with metastatic disease.

In Chapter 8, the prognostic value of the relatively new immunohistochemical marker L1CAM in endometrioid endometrial carcinoma is investigated.

In Chapter 9, findings are discussed in the light of the current understanding of the pathogenesis of tumor progression in (endometrioid) endometrial carcinomas, and the possibilities this understanding gives us to predict the clinical course of endometrial carcinoma patients.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69-90. Epub 2011/02/08.
2. www.oncoline.nl. 2011.
3. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Int J Gynaecol Obstet. 2009;105(2):103-4. Epub 2009/04/16.
4. Silverberg SG, Mutter GL, Kurman RJ, Kubik-Huch RA, Nogales F, Tavassoli FA. Tumors of the uterine corpus: epithelial tumors and related lesions. In: Tavassoli FA, Stratton FR, editors. WHO classification of tumors: pathology and genetics of tumors of the breast and female genital organs: World Health Organisation; 2003. p. 221-32.
5. Amant F, Mirza MR, Creutzberg CL. Cancer of the corpus uteri. Int J Gynaecol Obstet. 2012;119 Suppl 2:S110-7. Epub 2012/10/04.
6. Sivridis E, Giatromanolaki A. New insights into the normal menstrual cycle-regulatory molecules. Histol Histopathol. 2004;19(2):511-6. Epub 2004/03/17.
7. Gol K, Saracoglu F, Ekici A, Sahin I. Endometrial patterns and endocrinologic characteristics of asymptomatic menopausal women. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology. 2001;15(1):63-7. Epub 2001/04/11.
8. Bonnar J, Kraszewski A, Davis WB. Incidental pathology at vaginal hysterectomy for genital prolapse. The Journal of obstetrics and gynaecology of the British Commonwealth. 1970;77(12):1137-9. Epub 1970/12/01.
9. Mahajan G, Kotru M, Batra M, Gupta A, Sharma S. Usefulness of histopathological examination in uterine prolapse specimens. The Australian & New Zealand journal of obstetrics & gynaecology. 2011;51(5):403-5. Epub 2011/08/04.
10. Yin H, Mittal K. Incidental findings in uterine prolapse specimen: frequency and implications. Int J Gynecol Pathol. 2004;23(1):26-8. Epub 2003/12/12.
11. Sivridis E, Giatromanolaki A. Proliferative activity in postmenopausal endometrium: the lurking potential for giving rise to an endometrial adenocarcinoma. J Clin Pathol. 2004;57(8):840-4. Epub 2004/07/29.
12. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol. 1983;15(1):10-7. Epub 1983/02/01.
13. Sherman ME. Theories of endometrial carcinogenesis: a multidisciplinary approach. Mod Pathol. 2000;13(3):295-308. Epub 2000/04/11.
14. Engelsen IB, Akslen LA, Salvesen HB. Biologic markers in endometrial cancer treatment. APMIS. 2009;117(10):693-707. Epub 2009/09/25.
15. Prat J, Gallardo A, Cuatrecasas M, Catasus L. Endometrial carcinoma: pathology and genetics. Pathology. 2007;39(1):72-87. Epub 2007/03/17.
16. Kitchener HC, Trimble EL, Endometrial Cancer Working Group of the Gynecologic Cancer I. Endometrial cancer state of the science meeting. Int J Gynecol Cancer. 2009;19(1):134-40. Epub 2009/03/05.

17. McConechy MK, Ding J, Cheang MC, Wiegand KC, Senz J, Tone AA, et al. Use of mutation profiles to refine the classification of endometrial carcinomas. J Pathol. 2012;228(1):20-30. Epub 2012/06/02.
18. Sivridis E, Giatromanolaki A. The pathogenesis of endometrial carcinomas at menopause: facts and figures. J Clin Pathol. 2011;64(7):553-60. Epub 2011/02/19.
19. Nout RA, Bosse T, Creutzberg CL, Jurgenliemk-Schulz IM, Jobsen JJ, Lutgens LC, et al. Improved risk assessment of endometrial cancer by combined analysis of MSI, PI3K-AKT, Wnt/beta-catenin and P53 pathway activation. Gynecol Oncol. 2012;126(3):466-73. Epub 2012/05/23.
20. Lax SF. Molecular genetic changes in epithelial, stromal and mixed neoplasms of the endometrium. Pathology. 2007;39(1):46-54. Epub 2007/03/17.
21. Sivridis E, Fox H, Buckley CH. Endometrial carcinoma: two or three entities? Int J Gynecol Cancer 1998;8:183-8.
22. Benedetti Panici P, Basile S, Maneschi F, Alberto Lissoni A, Signorelli M, Scambia G, et al. Systematic pelvic lymphadenectomy vs. no lymphadenectomy in early-stage endometrial carcinoma: randomized clinical trial. J Natl Cancer Inst. 2008;100(23):1707-16. Epub 2008/11/27.
23. Kitchener H, Swart AM, Qian Q, Amos C, Parmar MK. Efficacy of systematic pelvic lymphadenectomy in endometrial cancer (MRC ASTEC trial): a randomised study. Lancet. 2009;373(9658):125-36. Epub 2008/12/17.
24. Dowdy SC, Mariani A. Lymphadenectomy in endometrial cancer: when, not if. Lancet. 2010;375(9721):1138-40. Epub 2010/03/02.
25. Roelofsen T, van Ham MA, de Hullu JA, Massuger LF. Clinical management of uterine papillary serous carcinoma. Expert review of anticancer therapy. 2011;11(1):71-81. Epub 2010/12/21.
26. Creutzberg CL, van Putten WL, Koper PC, Lybeert ML, Jobsen JJ, Warlam-Rodenhuis CC, et al. Surgery and postoperative radiotherapy versus surgery alone for patients with stage-1 endometrial carcinoma: multicentre randomised trial. PORTEC Study Group. Post Operative Radiation Therapy in Endometrial Carcinoma. Lancet. 2000;355(9213):1404-11. Epub 2000/05/03.
27. Nout RA, Smit VT, Putter H, Jurgenliemk-Schulz IM, Jobsen JJ, Lutgens LC, et al. Vaginal brachytherapy versus pelvic external beam radiotherapy for patients with endometrial cancer of high-intermediate risk (PORTEC-2): an open-label, non-inferiority, randomised trial. Lancet. 2010;375(9717):816-23. Epub 2010/03/09.
28. Randall ME, Filiaci VL, Muss H, Spirtos NM, Mannel RS, Fowler J, et al. Randomized phase III trial of whole-abdominal irradiation versus doxorubicin and cisplatin chemotherapy in advanced endometrial carcinoma: a Gynecologic Oncology Group Study. J Clin Oncol. 2006;24(1):36-44. Epub 2005/12/07.
29. Dijkhuizen FP, Mol BW, Brolmann HA, Heintz AP. The accuracy of endometrial sampling in the diagnosis of patients with endometrial carcinoma and hyperplasia: a meta-analysis. Cancer. 2000;89(8):1765-72. Epub 2000/10/24.
30. Bonadona V, Bonaiti B, Olschwang S, Grandjouan S, Huiart L, Longy M, et al. Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. JAMA. 2011;305(22):2304-10. Epub 2011/06/07.

31. Eltabbakh GH, Shamonki J, Mount SL. Surgical stage, final grade, and survival of women with endometrial carcinoma whose preoperative endometrial biopsy shows well-differentiated tumors. Gynecol Oncol. 2005;99(2):309-12. Epub 2005/07/12.
32. Mitchard J, Hirschowitz L. Concordance of FIGO grade of endometrial adenocarcinomas in biopsy and hysterectomy specimens. Histopathology. 2003;42(4):372-8. Epub 2003/03/26.
33. Traen K, Holund B, Mogensen O. Accuracy of preoperative tumor grade and intraoperative gross examination of myometrial invasion in patients with endometrial cancer. Acta obstetricia et gynecologica Scandinavica. 2007;86(6):739-41. Epub 2007/05/24.
34. DuBeshter B, Deuel C, Gillis S, Glantz C, Angel C, Guzick D. Endometrial cancer: the potential role of cervical cytology in current surgical staging. Obstet Gynecol. 2003;101(3):445-50. Epub 2003/03/15.
35. Brown AK, Gillis S, Deuel C, Angel C, Glantz C, Dubeshter B. Abnormal cervical cytology: a risk factor for endometrial cancer recurrence. Int J Gynecol Cancer. 2005;15(3):517-22. Epub 2005/05/11.
36. Fukuda K, Mori M, Uchiyama M, Iwai K, Iwasaka T, Sugimori H, et al. Preoperative cervical cytology in endometrial carcinoma and its clinicopathologic relevance. Gynecol Oncol. 1999;72(3):273-7. Epub 1999/03/04.
37. Todo Y, Minobe S, Okamoto K, Takeda M, Ebina Y, Watari H, et al. Cytological features of cervical smears in serous adenocarcinoma of the endometrium. Jpn J Clin Oncol. 2003;33(12):636-41. Epub 2004/02/11.
38. Larson DM, Johnson KK, Reyes CN, Jr., Broste SK. Prognostic significance of malignant cervical cytology in patients with endometrial cancer. Obstet Gynecol. 1994;84(3):399-403. Epub 1994/09/01.
39. Haldorsen IS, Berg A, Werner HM, Magnussen IJ, Helland H, Salvesen OO, et al. Magnetic resonance imaging performs better than endocervical curettage for preoperative prediction of cervical stromal invasion in endometrial carcinomas. Gynecol Oncol. 2012;126(3):413-8. Epub 2012/05/23.
40. Cade TJ, Quinn MA, McNally OM, Neesham D, Pyman J, Dobrotwir A. Predictive value of magnetic resonance imaging in assessing myometrial invasion in endometrial cancer: is radiological staging sufficient for planning conservative treatment? Int J Gynecol Cancer. 2010;20(7):1166-9. Epub 2011/04/19.
41. Mariani A, Dowdy SC, Cliby WA, Gostout BS, Jones MB, Wilson TO, et al. Prospective assessment of lymphatic dissemination in endometrial cancer: a paradigm shift in surgical staging. Gynecol Oncol. 2008;109(1):11-8. Epub 2008/02/29.
42. Moore RG, Brown AK, Miller MC, Badgwell D, Lu Z, Allard WJ, et al. Utility of a novel serum tumor biomarker HE4 in patients with endometrioid adenocarcinoma of the uterus. Gynecol Oncol. 2008;110(2):196-201. Epub 2008/05/23.
43. Angioli R, Miranda A, Aloisi A, Montera R, Capriglione S, De Cicco Nardone C, et al. A critical review on HE4 performance in endometrial cancer: where are we now? Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2013. Epub 2013/09/27.

44. Saarelainen SK, Peltonen N, Lehtimaki T, Perheentupa A, Vuento MH, Maenpaa JU. Predictive value of serum human epididymis protein 4 and cancer antigen 125 concentrations in endometrial carcinoma. Am J Obstet Gynecol. 2013;209(2):142 e1-6. Epub 2013/04/16.
45. Ali A, Black D, Soslow RA. Difficulties in assessing the depth of myometrial invasion in endometrial carcinoma. Int J Gynecol Pathol. 2007;26(2):115-23. Epub 2007/04/07.
46. Chattopadhyay S, Galaal KA, Patel A, Fisher A, Nayar A, Cross P, et al. Tumour-free distance from serosa is a better prognostic indicator than depth of invasion and percentage myometrial invasion in endometrioid endometrial cancer. BJOG : an international journal of obstetrics and gynaecology. 2012;119(10):1162-70. Epub 2012/07/19.
47. Kondalsamy-Chennakesavan S, van Vugt S, Sanday K, Nicklin J, Land R, Perrin L, et al. Evaluation of tumor-free distance and depth of myometrial invasion as prognostic factors for lymph node metastases in endometrial cancer. Int J Gynecol Cancer. 2010;20(7):1217-21. Epub 2011/04/19.
48. Schwab KV, O'Malley DM, Fowler JM, Copeland LJ, Cohn DE. Prospective evaluation of prognostic significance of the tumor-free distance from uterine serosa in surgically staged endometrial adenocarcinoma. Gynecol Oncol. 2009;112(1):146-9. Epub 2008/10/22.
49. Oreskovic S, Babic D, Kalafatic D, Barisic D, Beketic-Oreskovic L. A significance of immunohistochemical determination of steroid receptors, cell proliferation factor Ki-67 and protein p53 in endometrial carcinoma. Gynecol Oncol. 2004;93(1):34-40. Epub 2004/03/30.
50. Iwai K, Fukuda K, Hachisuga T, Mori M, Uchiyama M, Iwasaka T, et al. Prognostic significance of progesterone receptor immunohistochemistry for lymph node metastases in endometrial carcinoma. Gynecol Oncol. 1999;72(3):351-9. Epub 1999/03/04.
51. Salvesen HB, Iversen OE, Akslen LA. Prognostic significance of angiogenesis and Ki-67, p53, and p21 expression: a population-based endometrial carcinoma study. J Clin Oncol. 1999;17(5):1382-90. Epub 1999/05/20.
52. Kothari R, Morrison C, Richardson D, Seward S, O'Malley D, Copeland L, et al. The prognostic significance of the triple negative phenotype in endometrial cancer. Gynecol Oncol. 2010;118(2):172-5. Epub 2010/07/08.
53. Mariani A, Sebo TJ, Webb MJ, Riehle D, Katzmann JA, Keeney GL, et al. Molecular and histopathologic predictors of distant failure in endometrial cancer. Cancer detection and prevention. 2003;27(6):434-41. Epub 2003/12/04.
54. Ellis PE, Ghaem-Maghami S. Molecular characteristics and risk factors in endometrial cancer: what are the treatment and preventative strategies? Int J Gynecol Cancer. 2010;20(7):1207-16. Epub 2011/04/19.
55. Salvesen HB, Das S, Akslen LA. Loss of nuclear p16 protein expression is not associated with promoter methylation but defines a subgroup of aggressive endometrial carcinomas with poor prognosis. Clin Cancer Res. 2000;6(1):153-9. Epub 2000/02/03.
56. Ignatov A, Bischoff J, Schwarzenau C, Krebs T, Kuester D, Herrmann K, et al. P16 alterations increase the metastatic potential of endometrial carcinoma. Gynecol Oncol. 2008;111(2):365-71. Epub 2008/09/16.
57. Saegusa M, Hashimura M, Suzuki E, Yoshida T, Kuwata T. Transcriptional up-regulation of Sox9 by NF-kappaB in endometrial carcinoma cells, modulating cell proliferation through alteration in

the p14(ARF)/p53/p21(WAF1) pathway. The American journal of pathology. 2012;181(2):684-92. Epub 2012/06/16.

58. Trovik J, Wik E, Stefansson IM, Marcickiewicz J, Tingulstad S, Staff AC, et al. Stathmin overexpression identifies high-risk patients and lymph node metastasis in endometrial cancer. Clin Cancer Res. 2011;17(10):3368-77. Epub 2011/01/19.
59. Fogel M, Gutwein P, Mechtersheimer S, Riedle S, Stoeck A, Smirnov A, et al. L1 expression as a predictor of progression and survival in patients with uterine and ovarian carcinomas. Lancet. 2003;362(9387):869-75. Epub 2003/09/19.
60. Fogel M, Huszar M, Altevogt P, Ben-Arie A. L1 (CD171) as a novel biomarker for ovarian and endometrial carcinomas. Expert Rev Mol Diagn. 2004;4(4):455-62. Epub 2004/07/01.
61. Huszar M, Pfeifer M, Schirmer U, Kiefel H, Konecny GE, Ben-Arie A, et al. Up-regulation of L1CAM is linked to loss of hormone receptors and E-cadherin in aggressive subtypes of endometrial carcinomas. J Pathol. 2010;220(5):551-61. Epub 2010/01/16.
62. Zeimet AG, Reimer D, Huszar M, Winterhoff B, Puistola U, Abdel Azim S, et al. L1CAM in Early-Stage Type I Endometrial Cancer: Results of a Large Multicenter Evaluation. J Natl Cancer Inst. 2013. Epub 2013/06/20.
63. Darvishian F, Hummer AJ, Thaler HT, Bhargava R, Linkov I, Asher M, et al. Serous endometrial cancers that mimic endometrioid adenocarcinomas: a clinicopathologic and immunohistochemical study of a group of problematic cases. Am J Surg Pathol. 2004;28(12):1568-78. Epub 2004/12/04.
64. Colas E, Pedrola N, Devis L, Ertekin T, Campoy I, Martinez E, et al. The EMT signaling pathways in endometrial carcinoma. Clinical & translational oncology : official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico. 2012;14(10):715-20. Epub 2012/08/23.
65. Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Developmental cell. 2008;14(6):818-29. Epub 2008/06/10.
66. Bogenrieder T, Herlyn M. Axis of evil: molecular mechanisms of cancer metastasis. Oncogene. 2003;22(42):6524-36. Epub 2003/10/07.
67. Mirantes C, Espinosa I, Ferrer I, Dolcet X, Prat J, Matias-Guiu X. Epithelial-to-mesenchymal transition and stem cells in endometrial cancer. Hum Pathol. 2013;44(10):1973-81. Epub 2013/07/13.
68. Fidler IJ, Poste G. The “seed and soil” hypothesis revisited. The lancet oncology. 2008;9(8):808. Epub 2008/08/02.
69. Topolovec Z, Corusic A, Babic D, Mrcela M, Sijanovic S, Muller-Vranjes A, et al. Vascular endothelial growth factor and intratumoral microvessel density as prognostic factors in endometrial cancer. Collegium antropologicum. 2010;34(2):447-53. Epub 2010/08/12.
70. Pengchong H, Tao H. Expression of IGF-1R, VEGF-C and D2-40 and their correlation with lymph node metastasis in endometrial adenocarcinoma. Eur J Gynaecol Oncol. 2011;32(6):660-4. Epub 2012/02/18.

CHAPTER 2

HISTOPATHOLOGIC ASSESSMENT OF THE ENTIRE ENDOMETRIUM IN ASYMPTOMATIC WOMEN

Marjanka J.J.M. Mingels[1, x], MD, Yvette P. Geels[4, x], MD, Johanna M.A. Pijnenborg[2], MD, PhD, Anneke A. van der Wurff[3], MD, PhD, Angela A. G. van Tilborg[1,4],PhD, Maaike A.P.C. van Ham[1], MD, PhD, Leon F.A.G. Massuger[1], MD, PhD, Johan Bulten[4], MD, PhD.

[x]Marjanka J.J.M. Mingels and Yvette P. Geels contributed equally to this work.

[1]Department of Obstetrics and Gynecology, Radboud University Nijmegen Medical Centre, Nijmegen
[2]Department of Obstetrics and Gynecology, TweeSteden Hospital, Tilburg
[3]Department of Pathology, St. Elisabeth Hospital, Tilburg
[4]Department of Pathology, Radboud University Nijmegen Medical Centre, Nijmegen
The Netherlands

Hum Pathol. 2013 Oct;44(10):2293-301

Abstract

Objective: Knowledge on the nature of the endometrium in women without symptoms of endometrial disease is poor. Therefore, the aim of this prospective study was to describe the endometrium of a cohort of asymptomatic women.

Methods: The entire endometrium of pre- and postmenopausal women was embedded for histologic examination. All included patients underwent a hysterectomy on indication of uterovaginal prolapse, from July 2011 to October 2012, in three hospitals in the South of The Netherlands. Exclusion criteria were symptoms of postmenopausal vaginal blood loss, or premenopausal disordered vaginal bleeding.

Results: Sixty-eight women were included in the study, 48 women were postmenopausal and 20 were premenopausal. In the endometrium of ten women simple hyperplasia was found (15%), in one complex hyperplasia (2%), in two simple atypical hyperplasia (3%), in two complex atypical hyperplasia (3%), and in two a small focus of intramucosal endometrioid endometrial carcinoma (3%). In general, the endometrium was heterogenous and the majority of the lesions were not present in the entire endometrium.

Conclusion: after examining the entire endometrium, a remarkable high prevalence of endometrial pathology was found in asymptomatic women. The clinical meaning of these lesions is not yet clear, but endometrial pathology may frequently exist without symptoms.

Introduction

Endometrial carcinoma is the most common gynecologic malignancy in the Western population.[1] Endometrial carcinogenesis is thought to be a combination of genetic predisposition and environmental influences.[2] The majority is of endometrioid histology and arises in hyperplastic endometrium. In this type of endometrial carcinoma unopposed estrogenic stimulation leads to orderly progression from endometrial proliferation to hyperplasia, atypical hyperplasia, and finally to endometrioid endometrial carcinoma.[3] In about a quarter of women diagnosed with endometrial atypical hyperplasia development into endometrioid endometrial carcinoma occurs, within a time period of around four years.[4,5]

During the reproductive years, the endometrium is dynamic and undergoes hundreds of cycles of proliferation, differentiation and shedding. In premenopausal women the endometrium is expected to be proliferative or secretory, depending on the phase of the menstrual cycle. However, in postmenopausal women it is expected that the decline of estrogen production of the ovaries will result in atrophic endometrium.[6] However, excessive and unopposed estrogenic stimulation after menopause is described, and an unspecified proportion of menopausal women retain a weak proliferative pattern for many years.[7]

So far, the prevalence of endometrial lesions is mainly investigated in symptomatic women with abnormal bleeding. A limited number of studies investigated endometrial tissue in asymptomatic women. Based on review of biopsies, dilatation & curettage tissues or pathology reports from hysterectomies, hyperplasia was reported in 0.6-5.5%, atypical hyperplasia in 0.5-1.1% and carcinoma in 0.3-0.5% of the reviewed cases.[8-13] Yet, these studies are limited by the fact that endometrial sections of the hysterectomies were not reviewed, and embedding of the endometrium was performed according to routine protocol, including only one or two sections of the endometrium. It is known from literature that endometrial pathology can present focally, and is therefore easily missed if not the entire endometrium is embedded and reviewed.[14] Hence, routine sampling leaves the possibility that endometrial pathology will be missed.

The aim of the current study is to improve knowledge on the nature of the endometrium in pre- and postmenopausal women without symptoms of endometrial disease. Therefore, the prevalence of endometrial pathology is determined in asymptomatic patients who received

a hysterectomy for uterovaginal prolapse. The endometrium was entirely embedded in all cases for extensive histologic assessment.

Materials and Methods

Patient selection

In this prospective, multicentre study, women were included that were treated for uterovaginal prolapse with a vaginal, laparoscopically assisted, or abdominal, hysterectomy. All women were treated in the Radboud University Nijmegen Medical Centre from July 2011 to October 2012, or in the TweeSteden Hospital, and St. Elisabeth Hospital, Tilburg, from February 2012 to October 2012. Exclusion criteria were uterus myomatosus, abdominal pain, or any signs or symptoms of endometrial disease (menorrhagia, metrorrhagia, or postmenopausal vaginal bleeding). Of the included women, clinical data including age, body mass index, menopausal status, parity, medical history, and use of hormone replacement therapy were extracted from the medical records.

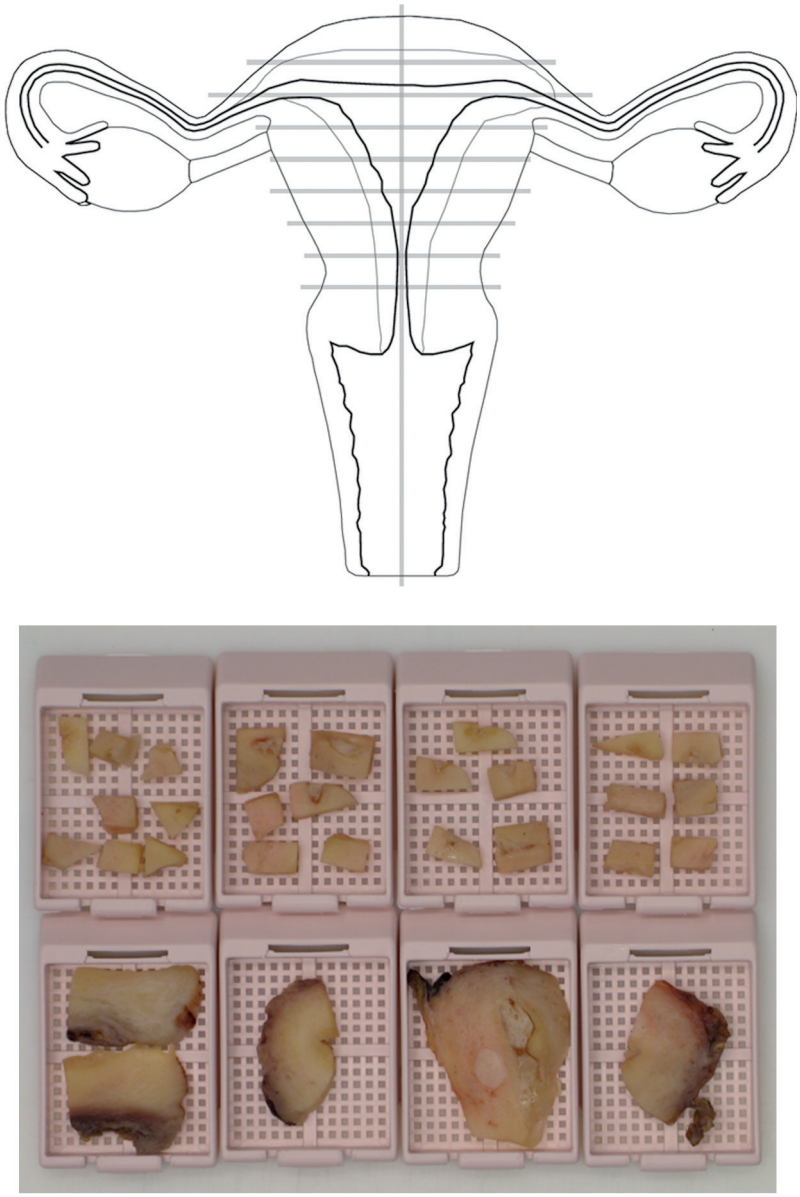
Sampling of the endometrium

After formalin fixation overnight, the uterus was sectioned for diagnostic purposes following routine protocol, including one or two endometrial sections for histologic assessment. The diagnostic process was completed by a pathologist, before the remaining endometrium was entirely sampled according to the SEE-End protocol (Sectioning and Extensively Examining the Endometrium) shown in Figure 1. The endometrium was cut transversely at 2 mm intervals before embedding in paraffin. All sections were stained with hematoxylin and eosin for histologic examination.

Viewing and reviewing of the endometrium

All endometrial sections were reviewed blindly by three of the authors (MM, YG, JB). In case of discrepancies, the case was discussed by all three authors and consensus was reached. Review was performed systematically by checking the following items: the nature of the endometrium, the presence of additional pathology in the endometrium (e.g. polyp, endometrial intra-epithelial carcinoma, or carcinoma), the presence of adenomyosis or leiomyomas, and measurement of endometrial and myometrial thickness.

Figure 1: Sampling of the endometrium according to the SEE-END protocol.



The endometrium was sampled entirely embedded following the SEE-End protocol (Sectioning and Extensively Examining the Endometrium). After completing the diagnostic process, the remaining endometrium was sectioned in transverse direction with intervals of 2 mm. Redundant myometrium was removed to enable the display of several endometrial sections in one section.

The endometrium was grouped in eight categories: proliferative endometrium, secretory endometrium, atrophy, disordered proliferative endometrium, simple hyperplasia (SH), complex hyperplasia (CH), simple atypical hyperplasia (SAH), complex atypical hyperplasia (CAH), and endometrial carcinoma. The categories simple and complex hyperplasia, simple and complex atypical hyperplasia and carcinoma were considered as endometrial pathology. Atrophic endometrium was defined as shallow endometrium with a thin basalis, and with a few tubular glands lined by inactive epithelium.[6] Proliferative or secretory endometrium was defined as widely spread, sometimes tortuous, tubular glands that show mitotic activity, pseudostratification of the nuclei, and abundant stroma.[6] Proliferation in the endometrium of postmenopausal patients is defined as disordered proliferative. This diagnosis was considered when some of the glands showed proliferative activity, and the gland:stroma ratio was slightly increased, but did not meet the criteria for hyperplasia.[14,15] Endometrial hyperplasia was defined as proliferation of glands with an increase in gland:stroma ratio of 3:1 and a variety of abnormal architectural patterns.[14] Cytologic atypia was defined as enlarged, rounded, polymorphic nuclei with loss of polarity, prominent nucleoli, chromatin clumbing, and an increased nuclear to cytoplasmic ratio.[15] Hyperplasia was categorized according to the World Health Organization (WHO) classification system for hyperplasia, which is based on the study of Kurman and colleagues.[4] When focal areas of hyperplasia or disordered proliferation were identified, the percentages of the entire endometrium containing this pathologic feature was estimated. The diagnosis of hyperplasia was only made when present in more than 10% of the total endometrial surface. Endometrial thickness was measured in all areas containing a pathologic lesion and in all areas with a non-pathologic diagnosis of the endometrium to enable comparison. At least two measurements were performed for each specific area of the endometrium.

Statistical analysis

For comparison between the groups of women with or without endometrial pathology the Pearson's chi-Square (χ^2) test, or the Fisher's exact test, when appropriate, were used. For comparison of continuous variables between both groups the Mann-Whitney *U* test was used.

Ethical committee approval

The study protocol was approved to be in accordance with the applicable rules concerning the review of research ethics committees and informed consent by the Research Ethics Committee of the Radboud University Nijmegen Medical Centre, and the Medical Ethical Test Committee of both the Elisabeth Hospital, and the TweeSteden Hospital, Tilburg.

Results

Patient characteristics

A total of 68 women who underwent a hysterectomy for uterovaginal prolapse were included in the study. Clinical characteristics are shown in Table 1. The mean age was 61 years (range 33-89) and 71% (48/68) was postmenopausal. In the vast majority of the women a vaginal hysterectomy was performed, and in a few an abdominal hysterectomy. The mean body mass index of these women was 25.9 kg/m^2 (range 19.6-39.8), and a minority had comorbidity like diabetes mellitus or hypertension. Twelve per cent (8/68) used hormone replacement therapy, estrogen suppletion opposed with progesterone, for menopausal complaints. None of the women had a history of tamoxifen use. Further, 9% (6/68) of the women used contraceptives, three used an intra-uterine device and three oral contraceptives. The mean number of deliveries in the studied group was 2.7 (range 1-8). None of the women were diagnosed with a malignancy or premalignancy in the past.

Histology

The mean number of endometrial sections available for review, after applying the SEE-End protocol, was 6, including a mean number of two sections embedded for standard diagnostic examination. (Sections contained several small areas with endometrial tissue for histologic examination.) Results of assessment of the endometrial sections are shown in Table 2. Examples of all diagnosed categories in the endometrium are shown in Figure 2. Endometrial pathology was observed in 25% (17/68) of the reviewed cases: simple hyperplasia in 15% (10/68), complex hyperplasia 2% (1/68), simple atypical hyperplasia in 3% (2/68), complex atypical hyperplasia in 3% (2/68) and endometrioid endometrial carcinoma in 3% (2/68) of cases. The remaining 75% of cases were diagnosed without endometrial pathology. Proliferative endometrium was found in 15% (10/68) of cases, secretory endometrium in

Table 1: Clinical characteristics of all included women (N=68).

Characteristic	Mean/N (range/%)
Age (years) (N=68)	60.5 (33-89)
BMI (kg/m^2) (N=41)	25.9 (19.6-39.8)
Parity (N=47)	2.7 (1.0-8.0)
Menopausal Status	
Premenopausal	20 (29.4)
Postmenopausal	48 (70.6)
Diabetes Mellitus	
No	58 (85.3)
Yes	6 (8.8)
Unknown	4 (5.9)
Hypertension	
No	49 (72.1)
Yes	15 (22.1)
Unknown	4 (5.8)
Use contraceptives	
Oral	3 (4.4)
Intra-Uterine Device	3 (4.4)
No or Unknown	62 (91.2)
Use HRT	
No	56 (83.3)
Yes	8 (11.8)
Unknown	4 (5.9)
Type of operation	
Vaginal Hysterectomy	62 (91.3)
Laparoscopic Assisted Hysterectomy	2 (2.9)
Abdominal Hysterectomy	2 (2.9)
Unknown	2 (2.9)

7% (5/68), and atrophic in 46% (31/68). Further, in 17 (25%) cases a polyp was found in the endometrium. One of these polyps was diagnosed with atypical hyperplasia, that was located both in the polyp as well as in the adjacent endometrial tissue. In all other polyps no atypical hyperplasia was identified.

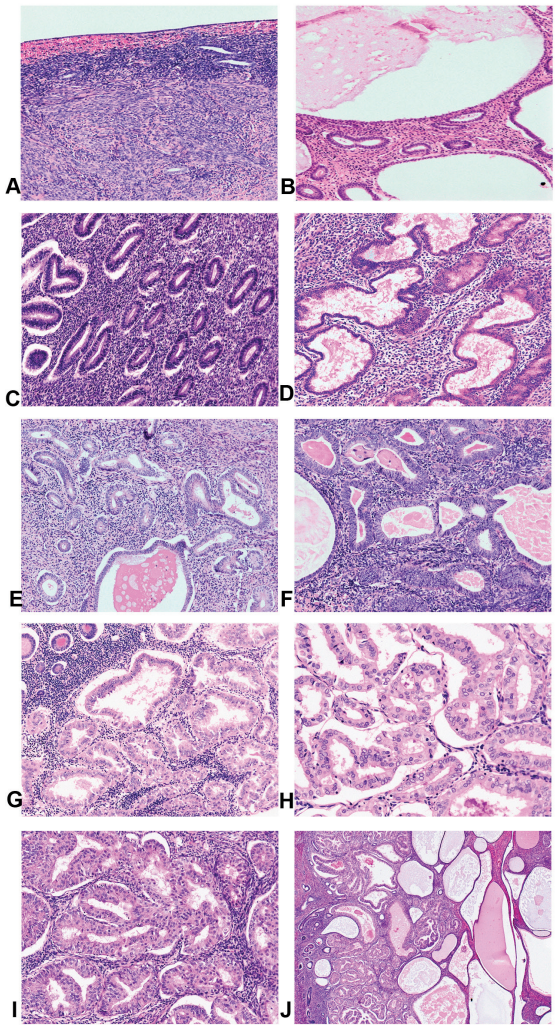
Results were specified for premenopausal and postmenopausal women (Table 2). In the postmenopausal women 10% (5/48) showed disordered proliferative endometrium. Of the premenopausal women 20% (4/20) had atrophic endometrium, and all four used contraceptives.

Table 2: Pathologic characteristics after review for the total group (N=68), and for premenopausal women (n=20) compared with postmenopausal women (N=48).

Characteristic	Total (N=68)	Premenopausal (N=20)	Postmenopausal (N=48)
	N (%)	N (%)	N (%)
Endometrial diagnosis			
Proliferative	10 (14.7)	10 (50.0)	-
Secretory	5 (7.4)	5 (25.0)	-
Atrophic	31 (45.6)	4 (20.0)	27 (56.3)
Disordered proliferative	5 (7.4)	-	5 (10.4)
Simple hyperplasia (SH)	10 (14.7)	1 (5.0)	9 (18.8)
Complex hyperplasia (CH)	1 (1.5)	0 (0.0)	1 (2.1)
Simple atypical hyperplasia (SAH)	2 (2.9)	0 (0.0)	2 (4.2)
Complex atypical hyperplasia (CAH)	2 (2.9)	0 (0.0)	2 (4.2)
Endometrioid endometrial carcinoma (EEC)	2 (2.9)	0 (0.0)	2 (4.2)
Additional identified lesions			
Polyp			
No	51 (75)	18 (90.0)	23 (68.7)
Yes, benign	15 (22.1)	2 (10.0)	13 (27.1)
Yes, hyperplastic	2 (2.9)	0 (0.0)	2 (4.2)
Metaplasia			
No	52 (76.5)	17 (85.0)	35 (72.9)
Yes, tubal	13 (19.1)	3 (15.0)	10 (20.8)
Yes, clear cell	3 (4.4)	0 (0.0)	3 (6.2)
Adenomyosis			
No	56 (82.4)	17 (85.0)	39 (81.2)
Yes	12 (17.6)	3 (15.0)	9 (18.8)
Leiomyomas			
No	52 (76.5)	16 (80.0)	36 (75.0)
Yes	16 (23.5)	4 (20.0)	12 (25.0)

The prevalence of endometrial pathology was significantly different in postmenopausal and premenopausal women, 33% and 5% respectively. Furthermore, in the group of women with endometrial pathology 13% (2/15) used hormone replacement therapy. One of these two women had simple hyperplasia, and one had complex atypical hyperplasia. In the group of women without endometrial pathology also 12% used hormone replacement therapy

Figure 2: Examples of the categories of the endometrium.



The nature of the endometrium in this prospective cohort of uterovaginal prolapsed cases was diagnosed in the following categories: A) Atrophic endometrium (level of magnification: 10x). B) Cystic atrophic (level of magnification: 10x). C) Proliferative endometrium (level of magnification: 10x). D) Secretory endometrium (level of magnification: 10x). E) Disordered proliferative (DP) (level of magnification: 10x). F) Simple hyperplasia (SH) (level of magnification: 10x). G) Complex atypical hyperplasia (CAH) (level of magnification: 10x). H) Identical area with complex atypical hyperplasia (CH), as in picture G, but at a higher level of magnification (20x) to show the atypia. I) Focus of intramucosal carcinoma (level of magnification: 10x). J) Overview of endometrium with identical area with focus of intramucosal carcinoma as in I, but at lower level of magnification (2x). Adjacent to the focus of intramucosal carcinoma, areas with cystic atrophic endometrium to complex atypical hyperplasia.

(6/49). In addition, the presence of additional endometrial and myometrial pathology is shown in Table 2.

In a significant part of the women the endometrium was heterogeneous. In Table 3, an overview is given of what percentage of endometrial pathology covered the endometrial surface. In two women a focal area was identified with intramucosal endometrioid endometrial carcinoma. Both were located in a background of atypical hyperplasia and only

Table 3: Overview of the entire endometrium in women who underwent a hysterectomy for uterovaginal prolapse and in which endometrial pathology was identified.

Endometrial pathology		Preoperative Ultrasound	Carcinoma	Atypical hyperplasia	Hyperplasia	Disordered proliferation (DP)	Background endometrium
Endometrial lesions	Case No.	Endometrial Thickness	%	%	%	%	%
EEC	Case No. 22	Small polyp	5% EEC	25% SHA + 25% CAH	25% SH	20% DP	-
EEC	Case No. 57	≤4mm	5% EEC	45% CAH	40% CH		10% Atrophic
CAH	Case No. 38	NA	-	20% SHA + 20% CAH	-	-	60% Atrophic
CAH	Case No. 43	NA	-	10% SHA + 10% CAH	70% SH + 10% CH	-	-
SAH	Case No. 9	≤4mm	-	5% SAH	5% SH	90% DP	
SAH	Case No. 63	≤4mm	-	20% SAH	30% SH	-	50% Atrophic
CH	Case No. 2	NA	-	-	20% SH + 10% CH	70% DP	-
SH	Case No. 19	≤4mm	-	-	10% SH	70% DP	20% Atrophic
SH	Case No. 20	≤4mm	-	-	30% SH	-	70% Atrophic
SH	Case No. 32	≤4mm	-	-	10% SH	50% DP	40% Atrophic
SH	Case No. 37	≤4mm	-	-	30% SH	50% DP	20% Atrophic
SH	Case No. 39	≤4mm	-	-	20% SH	-	80% Atrophic
SH	Case No. 50	≤4mm	-	-	20% SH	-	80% Atrophic
SH	Case No. 58	NA	-	-	10% SH	90% DP	-
SH	Case No. 59	NA	-	-	20% SH	-	80% Atrophic
SH	Case No. 60	≤4mm	-	-	10% SH	40% DP	50% Atrophic
SH	Case No. 67	NA	-	-	20% SH	60% DP	20% Atrophic

Abbreviations: Endometrial entities: endometrioid endometrial carcinoma (EEC), complex atypical hyperplasia (CAH), simple atypical hyperplasia (SAH), simple hyperplasia (SH), complex hyperplasia (CH), Disordered Proliferative (DP), Not available (NA).

a small area was diagnosed as atrophic in one case and disordered proliferative in the other (Table 3).

Changes of endometrial diagnosis after review of the initial sections and the additional embedded endometrial sections

In 12% (8/68) of women the additional embedding of the entire endometrium resulted in the identification of more severe endometrial pathology. In five cases the primary sections revealed atrophic or disordered proliferative endometrium, whereas after viewing the extra embedded endometrium, sampled in accordance with the SEE-End protocol, the diagnosis was changed to simple hyperplasia in four cases and atypical hyperplasia in one case. In three cases, there was simple hyperplasia in the initial sections. However, after viewing the additional sections a diagnosis of complex atypical hyperplasia was made in one case, and a focus of intramucosal endometrioid endometrial carcinoma with adjacent atypical hyperplasia was identified in the two other cases. Both women with endometrial carcinoma were postmenopausal.

Note that in one case with a focus of intramucosal carcinoma, the pre-operative ultrasound of the uterus showed an intra-cavitary abnormality. A biopsy was performed, and this abnormality was diagnosed as a benign polyp. When we reviewed this particular case we confirmed the presence of a benign polyp. The complex atypical hyperplasia and focus of carcinoma in this case were identified in the adjacent endometrium and not in the polyp. In other cases, including the second case with a focus of carcinoma, no abnormalities were reported on the pre-operative ultrasound.

Comparison of patients without and with endometrial pathology

Women with and without endometrial pathology were compared with respect to clinical and pathologic characteristics (Table 4). Women with endometrial pathology revealed to be significantly older and more often postmenopausal compared to the cases without endometrial pathology. Furthermore, the mean number of deliveries was higher in patients with endometrial pathology. The mean body mass index of patients with endometrial pathology was not significantly higher compared to the patients without endometrial pathology (27.8 kg/m^2 (range 23.1-39.8) *vs*. 25.3 kg/m^2 (range 19.6-36.8); *P*=0.114*)*. Preoperative ultrasound results showed no difference between women identified with

Table 4: Comparison of clinicopathologic characteristics between women without endometrial pathology and women with endometrial pathology (SH, CH, SAH, CAH and EEC).

	No pathology (N=51)	Pathology (N=17)	
Characteristic	Mean/N (range/%)	mean/N (range/%)	*P*-value
Age	57.7 (33-89)	68.9 (51-85)	0.002 [a]
BMI	25.3 (19.6-36.8)	27.8 (23.1-39.8)	0.114 [a]
Parity	2.5 (1-8)	3.5 (1-7)	0.028 [a]
Menopausal status			
pre	19 (37.3)	1 (5.9)	0.015 [c]
post	32 (62.7)	16 (94.1)	
Diabetes Mellitus			
No	45 (91.8)	13 (86.7)	0.618 [c]
Yes	4 (8.2)	2 (13.3)	
Hypertension			
No	38 (77.6)	11 (73.3)	0.737 [c]
Yes	11 (22.4)	4 (26.7)	
Use Contraceptives			
No	14 (70.0)	4 (100.0)	0.539 [c]
Yes	6 (30.0)	0 (0.0)	
Use HRT			
No	43 (87.7)	13 (86.7)	0.645 [c]
Yes	6 (12.3)	2 (13.3)	
Additional identified lesions			
Polyp			
No	41 (80.4)	10 (58.8)	0.075 [b]
Yes	10 (19.6)	7 (41.2)	
Metaplasia			
No	40 (78.4)	12 (70.6)	0.509 [b]
Yes	11 (21.6)	5 (29.4)	
Adenomyosis			
No	46 (90.2)	10 (58.8)	0.003 [b]
Yes	5 (9.8)	7 (41.2)	
Leiomyomas			
No	40 (78.4)	12 (70.6)	0.509 [b]
Yes	11 (21.6)	5 (29.4)	
Thickness myometrium (mm) (N=46)	12.38 (8.0-19.0)	11.63 (8.0-14.0)	0.598 [a]
Thickness endometrium (mm) (N=67)	1.23 (0.2-4.0)	1.16 (0.7-2.5)	0.196 [a]

[a] Mann-Whitney *U* test, [b] Pearson Chi-Square, [c] Fisher's exact test

or without endometrial pathology. In only one case an endometrial thickness above the threshold level of 4 mm was measured on preoperative ultrasound and was diagnosed as proliferative after histologic assessment. Available results of preoperative ultrasound of women in whom endometrial pathology was identified are shown in Table 3.

Further, no difference was found between endometrial thickness of pathologic and non-pathologic endometrial lesions measured in histologic sections. The mean endometrial thickness of areas with (cystic) atrophic endometrium, (disordered) proliferative, simple (atypical) hyperplasia, complex (atypical) hyperplasia and carcinoma was 1.3 mm (range 0.3-4.0 mm), 0.8 (range 0.5-1.3 mm), 1.2 (range 0.6-2.2 mm), 1.3 (range 0.7-2.2 mm) and 1.2 (range 1.0-3.0 mm), respectively.

Discussion

In the current prospective study, we systematically sampled the entire endometrium of women who underwent a hysterectomy for uterovaginal prolapse. A surprisingly high prevalence of endometrial abnormalities was identified in these asymptomatic women. In 25% of the women endometrial pathology was found: hyperplasia showed to be present in 16%, atypical hyperplasia in 6%, and a small focus of intramucosal endometrioid endometrial carcinoma in 3%. The endometrial thickness measured in the endometrial sections was not increased when endometrial pathology was present, indicating that it is unlikely to find this pathology pre-operatively with ultrasound.

Few studies analyzed the endometrium in asymptomatic women and reported a prevalence of endometrial abnormalities in only 5%. In studies examining curettage material in asymptomatic women, a prevalence of hyperplasia was found of 0.6-4.9%, hyperplasia with atypia of 0.5-0.6%, and adenocarcinoma of 0.5%.[8,11,12] When analyzing the pathology reports of hysterectomy specimens removed for benign reasons, hyperplasia was found to be present in 1.2-5.5%, atypical hyperplasia in 0.5-1.1%, and adenocarcinoma in 0.3-0.5%.[9,10,13,16,17] Restricted endometrial sampling and not performing a review of the histologic assessment are both important limitations that can putatively explain the large difference in prevalence of endometrial lesions found in comparison to the current prospective study.

The current study demonstrates the heterogeneity of the endometrium. Endometrial abnormalities can easily be missed when performing only standard sampling of the

endometrium of around two sections. In eight women (12%) the more extended embedding of the endometrium resulted in a more severe diagnosis, that was exclusively present in the additional embedded sections. In two women we found a small focus of intramucosal endometrioid carcinoma, which was not present in the standard embedded sections. Noteworthy is that in these two women, a diagnosis of hyperplasia was already made on the primary endometrial sections. This indicates that more extended review of the endometrium might be preferable in uteri of (asymptomatic) women in whom hyperplasia is found in the primary embedded slides.

An important strength of this observational study is that it is prospective and multicentre. Limitations of the study are that the collection of the clinical data was retrospective, and therefore sometimes incomplete. Further, eight patients did use hormone replacement therapy. However, estrogen suppletion in these patients was opposed with progesterone, and the number of patients using hormone replacement therapy was equal in the group with and without endometrial pathology. All patients underwent a hysterectomy, so follow up of the endometrial lesions was not possible. The aim of the study was to analyze the appearance of the endometrium in hysterectomy tissue of a general population. However, it is not investigated if hysterectomy tissue of women with prolapse uteri is comparable with tissue from asymptomatic women in the general population.

Moreover, the diagnosis of simple and complex hyperplasia with or without atypia is moderately reproducible. It has been proposed by several authors to use the hyperplasia-EIN (endometrial intraepithelial neoplasia) classification system for the diagnosis of pathologic endometrial proliferation.[18] However, the reproducibility of the EIN system is a matter of debate as well.[19] Therefore, we used the WHO classification system that is widespread, well understood and based on the classification system of Kurman and colleagues.[4]

The current study provides more insight into the appearance of the endometrium in hysterectomy tissue of asymptomatic women. The patients included in our study already underwent a hysterectomy, making the clinical importance of exactly identifying all types of endometrial lesions for these cases questionable. However, based on the results, we might conclude that endometrial lesions are more common in asymptomatic women than thought until now. These results are in line with a study on tubal epithelial lesions in normal women, reporting common presence of hyperplasia and minor atypia in the tubal epithelium as well.[20] Moreover, Horwitz *et al*, in 1981 already observed a four times greater rate of

endometrial cancer at autopsy than during live, indicating that a proportion of endometrial cancers may exist without symptoms.[21]

The clinical significance of (focal) hyperplasia has not yet been determined.[14] We considered the endometrium hyperplastic if at least ten percent of the endometrium met the criteria for hyperplasia, but the definition and impact of focal hyperplasia remains a grey area. Atypical hyperplasia diagnosed in endometrial curettings develops into carcinoma in about 25%.[4] However, if and in which percentage focal atypical hyperplasia needs to be present before it might progress to malignant disease is unclear.

Furthermore, it is suggested in literature that a state of weak proliferation can be found in the endometrium in up to half of postmenopausal women.[7] A plausible explanation for this phenomenon is the gradually declining estrogen stimulation without the opposition of ovulation in the first years after menopause. The results of our study confirm that a significant part of the postmenopausal patients have some form of weak proliferative endometrium. Disordered proliferation is stated to be a clinically benign process, since progression to hyperplasia may occur, but progression to carcinoma is extremely rare.[14] In our study, we therefore did not consider disordered proliferative endometrium as a pathologic condition. Factors known to give an increased risk of endometrial hyperplasia are all linked to unopposed, increased or prolonged estrogen stimulation, like late menopause, obesity, nulliparity, anovulatory cycles, diabetes mellitus, use of hormonal replacement therapy, and treatment with tamoxifen.[2,23] In the cohort of this study, except for a significant more advanced age and less premenopausal patients in the group with endometrial pathology, there were no differences in clinical characteristics between the group with and without endometrial pathology. An explanation for why a part of the postmenopausal patients developed hyperplasia and a part did not, cannot be given based on these results.

In conclusion, in this descriptive study on histologic assessment of the entire endometrium of pre- and postmenopausal, asymptomatic women, we found a high number of women with endometrial pathology in especially the postmenopausal group. If hyperplasia is identified in primary endometrial sections, additional sampling of the endometrium is recommended to exclude presence of (pre)malignancy. Probably, there is a higher prevalence of endometrial pathology in asymptomatic women than we know of, based on the existing literature, and endometrial pathology may frequently exist without symptoms.

References

1. Global Cancer Facts & Figures. 2008.
2. Sherman ME. Theories of endometrial carcinogenesis: a multidisciplinary approach. Mod.Pathol. 2000;13: 295-308.
3. Kurman RJ, McConnell TG. Precursors of endometrial and ovarian carcinoma. Virchows Arch. 2010;456: 1-12.
4. Kurman RJ, Kaminski PF, Norris HJ. The behavior of endometrial hyperplasia. A long-term study of "untreated" hyperplasia in 170 patients. Cancer 1985;56: 403-12.
5. Montgomery BE, Daum GS, Dunton CJ. Endometrial hyperplasia: a review. Obstet.Gynecol.Surv. 2004;59: 368-378.
6. McCluggage WG. Benign Diseases of the Endometrium. In: Kurman RJ RB, Ellenson HL, editor. Blaustein's Pathology of the Female Genital Tract. Sixth Edition. New York: Springer; 2011, p. 305-358.
7. Sivridis E, Giatromanolaki A. Proliferative activity in postmenopausal endometrium: the lurking potential for giving rise to an endometrial adenocarcinoma. J Clin Pathol 2004;57: 840-4.
8. Archer DF, McIntyre-Seltman K, Wilborn WW, Jr., et al. Endometrial morphology in asymptomatic postmenopausal women. Am J Obstet Gynecol 1991;165: 317-20; discussion 320-2.
9. Bonnar J, Kraszewski A, Davis WB. Incidental pathology at vaginal hysterectomy for genital prolapse. J.Obstet.Gynaecol.Br.Commonw. 1970;77: 1137-1139.
10. Frick AC, Walters MD, Larkin KS, Barber MD. Risk of unanticipated abnormal gynecologic pathology at the time of hysterectomy for uterovaginal prolapse. Am.J.Obstet.Gynecol. 2010;202: 507-4.
11. Gol K, Saracoglu F, Ekici A, Sahin I. Endometrial patterns and endocrinologic characteristics of asymptomatic menopausal women. Gynecol.Endocrinol. 2001;15: 63-67.
12. Korhonen MO, Symons JP, Hyde BM, Rowan JP, Wilborn WH. Histologic classification and pathologic findings for endometrial biopsy specimens obtained from 2964 perimenopausal and postmenopausal women undergoing screening for continuous hormones as replacement therapy (CHART 2 Study). Am.J.Obstet.Gynecol. 1997;176: 377-380.
13. Mahajan G, Kotru M, Batra M, Gupta A, Sharma S. Usefulness of histopathological examination in uterine prolapse specimens. Aust N Z J Obstet Gynaecol 2011;51: 403-5.
14. Mills AM, Longacre TA. Endometrial hyperplasia. Semin Diagn Pathol 2010;27: 199-214.
15. Ellenson HL RB, Kurman RJ. Precursor lesions of endometrial carcinoma. In: Kurman RJ RB, Ellenson HL, editor. Blaustein's Pathology of the Female Genital Tract. Sixth Edition. New York: Springer; 2011, p. 359-91.
16. Yin H, Mittal K. Incidental findings in uterine prolapse specimen: frequency and implications. Int J Gynecol Pathol 2004;23: 26-8.
17. Salmon HA, Smith JH, Balsitis M. Is microscopic assessment of macroscopically normal hysterectomy specimens necessary? J Clin Pathol 2002;55: 67-8.
18. Mutter GL. Endometrial intraepithelial neoplasia (EIN): will it bring order to chaos? The Endometrial Collaborative Group. Gynecol Oncol 2000;76: 287-90.

19. Lacey JV, Jr., Mutter GL, Nucci MR, et al. Risk of subsequent endometrial carcinoma associated with endometrial intraepithelial neoplasia classification of endometrial biopsies. Cancer 2008;113: 2073-81.
20. Mingels MJ, Roelofsen T, van der Laak JA, et al. Tubal epithelial lesions in salpingo-oophorectomy specimens of BRCA-mutation carriers and controls. Gynecol Oncol 2012;127:88-93.
21. Horwitz RI, Feinstein AR, Horwitz SM, Robboy SJ. Necropsy diagnosis of endometrial cancer and detection-bias in case/control studies. Lancet 1981;2: 66-8.
22. Amant F, Mirza MR, Creutzberg CL. Cancer of the corpus uteri. Int J Gynaecol Obstet 2012;119 Suppl 2: S110-7.
23. Sturgeon SR, Sherman ME, Kurman RJ, et al. Analysis of histopathological features of endometrioid uterine carcinomas and epidemiologic risk factors. Cancer Epidemiol Biomarkers Prev 1998;7: 231-5.

CHAPTER 3

CERVICAL CYTOLOGY IN SEROUS AND ENDOMETRIOID ENDOMETRIAL CANCER

Thijs Roelofsen[1,x], MD; Yvette P. Geels[1,x], MD; Johanna M.A. Pijnenborg[2], MD, PhD; Maaike A.P.C. van Ham[1], MD, PhD; Saskia F. Zomer[3], MD; Johanna M. Wiersma van Tilburg[4], MD; Marc P.M.L. Snijders[5], MD, PhD; Albert G. Siebers[6], PhD; Johan Bulten[6], MD, PhD; Leon F.A.G. Massuger[1], MD, PhD.

[x] T. Roelofsen and Y.P. Geels contributed equally to this work.

[1]Department of Obstetrics and Gynecology, Radboud University Nijmegen Medical Centre, the Netherlands
[2]Department of Obstetrics and Gynecology, Twee Steden Hospital Tilburg, the Netherlands
[3]Department of Pathology, Canisius Wilhelmina Hospital, Nijmegen, the Netherlands
[4]Department of Pathology, Rijnstate Hospital, Arnhem, the Netherlands
[5]Department of Obstetrics and Gynecology, Canisius Wilhelmina Hospital, Nijmegen, the Netherlands
[6]Department of Pathology, Radboud University Nijmegen Medical Centre, the Netherlands

Int J Gynecol Pathol. 2013 Jul;32(4):390-8

Abstract

Objective: To determine the frequency of abnormal cervical cytology in preoperative cervical cytology of patients diagnosed with uterine papillary serous carcinoma (UPSC) and endometrioid endometrial carcinoma (EEC). In addition, associations between abnormal cervical cytology and clinicopathologic factors were evaluated.

Methods: In this multicentre study, EEC patients diagnosed at two hospitals from 1999 to 2009 and UPSC patients diagnosed at five hospitals from 1992 to 2009, were included. Revision of the histologic slides was performed systematically and independently by three gynecopathologists. Cervical cytology within six months before histopathologic diagnosis of endometrial carcinoma was available for 267 EEC and 80 UPSC patients. Cervical cytology with atypical, malignant, or normal endometrial cells in postmenopausal women was considered as abnormal cytology, specific for endometrial pathology.

Results: Abnormal cervical cytology was found in 87.5% of UPSC patients, compared to 37.8% in EEC patients. In UPSC, abnormal cytology was associated with extra-uterine spread of disease (P=0.043). In EEC, abnormal cytology was associated with cervical involvement (P=0.034). In both EEC and UPSC patients, abnormal cervical cytology was not associated with survival.

Conclusion: Abnormal cervical cytology was more frequently found in UPSC patients. It was associated with extra-uterine disease in UPSC patients, and with cervical involvement in EEC patients. More prospective research should be performed to assess the true clinical value of preoperative cervical cytology in endometrial cancer patients.

Introduction

Endometrial cancer is the most frequent malignancy of the female genital tract in the western world.[1] The majority of patients with endometrial cancer are diagnosed with endometrioid endometrial carcinoma (EEC), typically presenting at an early stage with an excellent prognosis; five-year overall survival rates of 70-80% have been reported.[2,3] In contrast, uterine papillary serous carcinoma (UPSC) represents only 10% of endometrial cancers, but accounts for up to 39% of all endometrial cancer deaths. UPSC is therefore recognized as an aggressive tumor.[4] Unlike its EEC counterpart, UPSC commonly presents with advanced stage of disease and poor prognosis, indicated by a five-year survival rate of only 18-45%.[4,5]

The treatment of endometrial cancer is primarily based on surgery, consisting of hysterectomy and bilateral salpingo-oophorectomy (BSO). There is no worldwide consensus whether pelvic and/or para-aortic lymphadenectomy should be performed as part of the staging procedure.[6,7] For EEC patients the extensiveness of surgery depends on the presence of risk factors for metastatic disease, like high tumor grade, deep myometrial invasion, and cervical involvement.[7] However, preoperative assessment of these factors remains a challenge. After histopathologic examination, only 8-13% of the EEC patients have cervical involvement, and about 7% will have extra-uterine disease at the time of diagnosis.[8,9] In contrast, in UPSC patients 55-87% have microscopic or macroscopic metastases outside the uterus at the time of diagnosis.[10,11] For these patients, debulking surgery and comprehensive surgical staging (including hysterectomy, BSO, bilateral pelvic lymph node dissection with para-aortic lymph node sampling and omental biopsy or omentectomy) have been suggested to more reliably determine stage of disease, to guide adjuvant treatment, and to improve survival.[12,14]

In asymptomatic women, cervical cytology appeared to be a poor screening tool for endometrial carcinoma because of its low sensitivity.[15,16] However, when normal or atypical endometrial cells are found in cervical cytology of postmenopausal women, it is predictive for endometrial pathology.[15,17,18] Furthermore, cervical cytology has shown to be of additional value for the prediction of cervical stroma involvement and lymph node metastases.[19-21] In addition, in patients with endometrial cancer, cervical cytology with atypical or malignant endometrial cells was associated with advanced stage of disease, high tumor grade and deep myometrial invasion.[22-26]

In patients with endometrial cancer, the frequency of atypical or malignant endometrial cells in preoperative cervical cytology varies from 31-50%.[22,24,25] It has been reported though, that in UPSC patients cervical cytology is more likely to contain atypical or malignant endometrial cells.[27] However, cervical cytology has only been investigated in cohorts with limited number of UPSC patients, and evidence for associations with poor prognostic factors in UPSC patients is lacking.[25,27-30]

The aim of this study was to evaluate the presence of shedded atypical endometrial cells in cervical cytology during the diagnostic and/or preoperative workup of patients with UPSC as compared to patients with EEC. Possible associations between abnormal cervical cytology and clinicopathologic variables were studied in both cohorts. Furthermore, we evaluated whether the presence of atypical endometrial cells in preoperative cervical cytology has prognostic significance for survival in UPSC and EEC patients.

Methods

Patient selection

The nationwide network and registry of histology and cytopathology in the Netherlands (Pathologisch Anatomisch Landelijk Geautomatiseerd Archief: PALGA)[31] was used to search for all patients with primary EEC, diagnosed and treated at the Radboud University Nijmegen Medical Centre or the Canisius Wilhelmina Hospital in the period between January 1999 and December 2009. Furthermore, this network was used to search for all patients diagnosed with UPSC in the same two hospitals and three additional hospitals (Rijnstate Hospital Arnhem, Gelderse Vallei Hospital Ede, and Maas Hospital Boxmeer) from January 1992 till December 2009. Patients were excluded in case of a second primary malignancy. Pathologic, medical and operative records of all patients were retrieved from the hospitals involved.

All histopathologic slides from surgery specimens were reviewed systematically by three expert pathologists (SZ, AW and JB). Review included tumor histology, tumor grade, depth of myometrial invasion, and the presence of lymphovascular space invasion (LVSI). As per definition, UPSC was considered to be grade 3 carcinoma.[32,33] Patients with UPSC were included when the carcinoma comprised at least 10% serous histology according to previously published criteria.[32,34] The included UPSC cases were defined as pure UPSC, with

the serous component comprising >75% of the total tumor, and mixed UPSC, with the serous component comprising 10-75% of the tumor.

Revised UPSC and EEC patients were included when cervical cytology was taken for the diagnostic and/or preoperative workup because of clinical suspicion for malignancy, within a time frame of 6 months before histopathologic diagnosis. To note, all included patients had cervical cytology taken before endometrial biopsy or dilatation and curettage. In case multiple cervical smears were taken during this six months' time interval, the smear with the most severe diagnosis was used for our analyses.

Cervical Cytology

PALGA was used to retrieve the complete cervical cytology history of each EEC and UPSC patient. This database has nationwide coverage from 1991 onwards, showing all surgical specimens and cervical cytology ever taken from each patient, both by the general practitioner and medical specialist.[31] Within our study period, cervical cytology was obtained using both conventional cytology and the more recently introduced liquid based cytology.[35] Liquid based cytology was introduced between 1996 and 2003 at the different pathology laboratories. Cervical cytology was screened and classified by cytotechnologists and approved by a pathologist according to the CISOE-A classification system, of which results are easily translatable to the various Bethesda 2001 subcategories.[36] CISOE-A explicitly specifies the presence of normal and abnormal endometrial cells. Cervical cytology was classified as normal if there were no endometrial cells present. Atypical or malignant endometrial cells that were diagnosed in diagnostic or preoperative cervical cytology was considered as an abnormal cytological result, indicating endometrial pathology. Cervical cytology showing normal endometrial cells was considered abnormal only in postmenopausal women. To note, atypical squamous or atypical glandular/endocervical cells were not considered as abnormal cytology.

Because of the retrospective study design, patients were staged according to the 1988 FIGO surgical staging system.[37] All patients underwent primary surgical treatment, except for four UPSC patients because of serious comorbidity (N=3) or because the patient refused treatment (N=1). These four patients remained to be included in our study to investigate the frequency of abnormal endometrial cells in preoperative cervical cytology and their

relationship with various clinicopathologic variables. However, these four UPSC patients did not receive optimal surgical and/or adjuvant treatment and were therefore excluded from further analyses on survival. Clinicopathologic data were collected regarding age, body mass index (BMI), FIGO stage, peritoneal cytology, lymph node metastases, cervical involvement, extra-uterine disease, histology, and tumor diameter. Extra-uterine spread of disease was defined as cervical involvement, nodal involvement, positive peritoneal cytology, and/or disease at any other site outside the uterus.

Statistical analysis

To analyze the correlation between different clinicopathologic variables with abnormal cervical cytology, univariable analyses were performed, with χ^2 or Fisher's exact test analyses for categorical variables, independent *t* test for continuous variables, and univariable logistic regression analyses when appropriate. Furthermore, to examine the effects of various clinicopathogic variables on progression free survival (PFS), univariable analyses were performed using the Cox proportional hazard method. PFS was defined as the time in months from initial surgery to the date of recurrence. In case of no recurrence, the date of last contact or death was used for censoring. All statistical analyses were performed using SPSS statistical software for windows, version 18.0 (SPSS, Inc, Chicago, IL). $P<0.05$ was considered statistically significant.

Results

Records of 141 UPSC patients and 353 EEC patients were retrieved from the hospitals involved. In the UPSC group, 26 patients were excluded because the serous component within the tumor comprised less than 10%, whereas 19 EEC patients were excluded because of (partly) non-endometrioid histology. Furthermore, from 35 UPSC patients and 67 EEC patients cervical cytology was either not taken for diagnostic/preoperative workup within 6 months prior to diagnosis, or unsatisfactory for diagnosis, and these patients were excluded. Thus, a total of 80 UPSC and 267 EEC patients comprised our study population.

Demographic and histopathologic characteristics of the UPSC and EEC patients are presented in Table 1. For UPSC patients, the median age at diagnosis was 72 years (range from 47 to 86), whereas the median age at diagnosis for EEC was 63 years (range from 38 to 92). Forty-

Table 1: Clinical and pathologic characteristics in patients with UPSC and EEC.

Variables	UPSC patients (N=80) Median (range)/N (%)	EEC patients (N=267) Median (range)/N (%)
Age at diagnosis (years)	72 (47-86)	63 (38-92)
BMI (kg/m^2)	27.0 (18.0-41.0)	28.3 (18.4-53.6)
FIGO Stage		
I	28 (35.0)	215 (80.5)
II	9 (11.3)	22 (8.2)
III	21 (26.3)	18 (6.7)
IV	22 (27.4)	12 (4.5)
Peritoneal Cytology		
Negative	42 (63.6)	189 (91.7)
Positive	24 (36.4)	17 (8.3)
Not sampled	14	61
Para-aortic and/or Pelvic Lymph nodes		
Negative	23 (63.9)	32 (91.4)
Positive	13 (36.1)	3 (8.6)
Not sampled[a]	44	232
Extra-uterine disease		
No	28 (35.0)	215 (80.5)
Yes	52 (65.0)	52 (19.5)
Cervical involvement		
No	46 (59.7)	234 (87.6)
Yes	31 (40.3)	33 (12.4)
Unknown	3	0
LVSI		
No	41 (53.9)	202 (78.9)
Yes	35 (46.1)	54 (21.1)
Unknown	4	11
Histology		
Pure histology	62 (77.5)	NA[$]
Mixed histology	18 (22.5)	
Tumor Grade		
1	0 (0.0)	115 (43.0)
2	0 (0.0)	110 (41.2)
3	80 (100.0)	42 (15.7)
Myometrial invasion		
≤1/2 myometrium	36 (48.0)	159 (59.6)
>1/2 myometrium	39 (52.0)	108 (40.4)
Unknown	5	0
Diameter of tumor (cm)	4.0 (0.5-10.0)	2.5 (0.2-8.0)
Median time of follow-up (months)	19 (1-163)	47 (0-126)

[a]Lymph node status assigned only by inspection/palpation at laparotomy or from imaging; FIGO: International Federation of Obstetrics and Gynecologists; LVSI: lymphovascular space invasion; UPSC: uterine papillary serous carcinoma; EEC: endometrioid endometrial carcinoma. [$]Not available: the variable 'histology' is a constant in EEC patients.

three (53.7%) UPSC patients were diagnosed with advanced stage (III-IV) disease, compared to 30 (11.2%) EEC patients. In 52 (65.0%) UPSC patients extra-uterine spread of disease was found, compared to 52 (19.5%) of the patients with EEC. UPSC patients, compared to EEC patients, more often had LVSI (46.1% and 21.1% respectively), cervical involvement (40.3% and 12.4% respectively) deep myometrial invasion (52.0% and 40.4% respectively), and a larger median tumor diameter (4.0 and 2.5 respectively). In 36 UPSC patients (45%) lymphadenectomy was performed of whom 36.1% had positive pelvic or para-aortic lymph nodes. To note, among UPSC patients without proper lymph node sampling, the majority had obvious disseminated disease based on macroscopic tumor deposits, bone or lung metastases or peritoneal disease. In EEC patients, lymph node sampling was omitted in cases without clinical suspicion of FIGO stage II or more, as recommended by the Dutch guidelines for endometrioid endometrial cancer treatment. Thirty-five EEC patients (13.1%) underwent lymphadenectomy of whom 8.6% had positive lymph nodes. The median follow-up time for UPSC patients was 19 months (range 1-163 months) and 47 months (range 0-126 months) for EEC patients. Thirty-seven UPSC patients (48.7%) and 34 EEC patients (14.8%) had recurrence of disease, resulting in a five-year PFS of 40.2% and 90.8% respectively.

Preoperative cervical cytology

The cervical cytology findings in both UPSC and EEC patients are listed in Table 2. The median time interval between initial cervical cytology and final histopathologic diagnosis for endometrial carcinoma was 1 month (range 0-6 months) for UPSC patients and 1.5 month (range 0-6 months) for EEC patients. In the group with 80 UPSC patients, 20 patients (25.0%) had atypical endometrial cells and 50 (62.5%) had malignant endometrial cells in their cervical cytology. Seven patients (8.8%) had normal cervical cytology preoperatively, and three patients (3.7%) had abnormal cervical cytology not specific for endometrial pathology: two cases with atypical glandular/endocervical epithelial cells and one case with atypical squamous epithelial cells. In total, 70 UPSC patients (87.5%) had abnormal cervical cytology, specific for endometrial pathology preoperatively (Table 2).

Within the group of 267 EEC patients, in 48 patients (18.0%) atypical endometrial cells were found in cervical cytology. In four patients (1.5%) normal endometrial cells were found, these four patients were postmenopausal. Forty-nine patients (18.4%) had malignant

Table 2: Preoperative cervical cytology findings within six months prior to diagnosis of UPSC or EEC.

	UPSC patients (N=80)		EEC patients (N=267)	
Pathology in cervical cytology	N (%)		N (%)	
Normal endometrial cells in smear	0	(0)	4	(1.5)
Atypical endometrial cells in smear	20	(25.0)	48	(18.0)
Malignant endometrial cells in smear	50	(62.5)	49	(18.4)
Total abnormal cervical cytology	**70**	**(87.5)**	**101**	**(37.8)**
Cervical cytology without pathology	7	(8.8)	163	(61.0)
Other[#] pathology in smear	3	(3.7)	3	(1.1)
Total normal cervical cytology	**10**	**(12.5)**	**166**	**(62.2)**

UPSC: uterine papillary serous carcinoma; EEC: endometrioid endometrial carcinoma; [#]Atypical squamous epithelial cells, or atypical glandular/endocervical epithelial cells.

endometrial cells in their preoperative cervical cytology. In 163 EEC patients (61.0%) normal cervical cytology was found preoperatively, and three patients (1.1%) had abnormal cytology findings not specific for endometrial pathology: two with atypical squamous cells, and one with atypical glandular/endocervical cells. In total 101 EEC patients (37.8%) had abnormal cervical cytology, specific for endometrial pathology, prior to their diagnosis.

Associations of abnormal cervical cytology with clinicopathologic findings

In UPSC patients, only extra-uterine spread of disease (P=0.043) was significantly associated with an increased frequency of abnormal cervical cytology (Table 3). Using univariable logistic regression, extra-uterine disease remained the only factor associated with abnormal cervical cytology (OR 5.11, 95% CI 1.02–28.36; data not shown). Advanced stage of disease was not associated with abnormal cervical cytology; 23 of 28 UPSC patients (82.1%) with stage I disease already had abnormal cervical cytology. In addition, patients lacking poor prognostic factors, like negative peritoneal cytology, absence of lymph node metastases, or no cervical involvement, had abnormal cervical cytology in 37 of 40 (92.5%), 20 of 21 (95.2%), and 41 of 46 (89.1%) cases, respectively. There was no significant difference in frequency of abnormal cervical cytology among pure UPSC and mixed UPSC patients. Other well-known prognostic factors such as myometrial invasion, LVSI, and tumor diameter, were also not associated with abnormal cervical cytology in UPSC patients.

Table 3: Associations preoperative cervical cytology findings with clinicopathologic variables in patients with UPSC and EEC.

Variable	Cervical cytology in UPSC patients‡			Cervical cytology in EEC patients‡		
	Normal (%)	Abnormal (%)	*P*-value	Normal (%)	Abnormal (%)	*P*-value
Mean Age (years)	75.7	70.5	0.140#	64.6	63.4	0.270#
Mean BMI (kg/m^2)	26.7	27.0	0.872#	28.9	30.5	0.092#
FIGO Stage						
I	5 (71.4)	23 (32.8)	0.151∞	139 (83.7)	76 (75.3)	0.392
II	0 (0.0)	7 (10.0)		12 (7.3)	10 (9.9)	
III	0 (0.0)	20 (28.6)		9 (5.4)	9 (8.9)	
IV	2 (28.6)	20 (28.6)		6 (3.6)	6 (5.9)	
Peritoneal cytology						
Negative	3 (60.0)	37 (62.7)	0.904∞	125 (92.6)	64 (90.1)	0.543
Positive	2 (40.0)	22 (37.3)		10 (7.4)	7 (9.9)	
Para-aortic and/or Pelvic Lymph nodes						
Negative	1 (50.0)	20 (62.5)	0.724∞	20 (100.0)	12 (80.0)	0.070∞
Positive	1 (50.0)	12 (37.5)		0 (0.0)	3 (20.0)	
Extra-uterine spreading						
No	5 (71.4)	23 (32.9)	**0.043**∞	139 (83.7)	76 (75.2)	0.089∞
Yes	2 (28.6)	47 (67.1)		27 (16.3)	25 (24.8)	
Cervical involvement						
No	5 (83.3)	41 (60.3)	0.265∞	151 (91.0)	83 (82.2)	**0.034**
Yes	1 (16.7)	27 (39.7)		15 (9.0)	18 (17.8)	
LVSI						
No	3 (42.9)	36 (54.5)	0.556∞	129 (81.6)	73 (74.5)	0.173
Yes	4 (57.1)	30 (45.5)		29 (18.4)	25 (25.5)	
Histology						
Pure histology	5 (71.4)	56 (80.0)	0.594∞	166 (100.0)	101 (100.0)	NA$
Mixed histology	2 (28.6)	14 (20.0)		0 (0.0)	0 (0.0)	
Tumor grade						
1	0 (0.0)	0 (0.0)	NA*	78 (47.0)	37 (36.6)	0.274
2	0 (0.0)	0 (0.0)		65 (39.2)	45 (44.6)	
3	7 (100.0)	70 (100.0)		23 (13.8)	19 (18.8)	
Myometrial invasion						
≤1/2 myometrium	2 (33.3)	32 (48.5)	0.477∞	98 (59.0)	61 (60.4)	0.826
>1/2 myometrium	4 (66.7)	34 (51.5)		68 (41.0)	40 (39.6)	
Mean Diameter of tumor (cm)	3.1	4.0	0.343#	2.9	2.6	0.236#

‡Adjusted for abnormal cytology specific for endometrial pathology; #Independent T-test was used for continues variables; ∞Fisher's exact test; *Not available: the variable 'tumor grade' is a constant in UPSC patients. $Not available: the variable 'histology' is a constant in EEC patients. LVSI: lymphovascular space invasion; UPSC: uterine papillary serous carcinoma; EEC: endometrioid endometrial carcinoma.

Table 4: Crude hazard ratios (HR) with 95% confidence interval (CI) of Progression Free Survival (PFS) by clinicopathological variable in UPSC and EEC patients, using univariable Cox regression.

		PFS-UPSC		PFS-EEC
Variable	N	HR (95% CI)	N	HR (95% CI)
Age at diagnosis (years)	76	1.00 (0.96-1.03)	283	1.03 (0.99-1.06)
BMI (kg/m^2)	68	0.93 (0.86-1.01)	242	0.99 (0.93-1.05)
FIGO Stage				
I – II	37	1.00 (reference)	251	1.00 (reference)
III – IV	39	**6.87 (3.22-14.67)**	32	**9.66 (4.91-19.01)**
Histology				
Pure	58	1.00 (reference)		NA[$]
Mixed	18	0.79 (0.36-1.73)		
Tumor grade				
Low (grade 1 - 2)		NA[*]	242	1.00 (reference)
High (grade 3)			41	**5.82 (2.95-11.48)**
Extra-uterine disease				
No	28	1.00 (reference)	212	1.00 (reference)
Yes	48	**6.67 (2.75-16.16)**	51	**6.75 (3.39-13.43)**
Cervical involvement				
No	46	1.00 (reference)	230	1.00 (reference)
Yes	29	1.84 (0.95-3.55)	33	**3.29 (1.53-7.10)**
Para-aortic and/or pelvic Lymph nodes				
Negative	21	1.00 (reference)	32	NA[#]
Positive	12	**3.96 (1.40-11.20)**	3	NA[#]
LVSI				
No	41	1.00 (reference)	219	1.00 (reference)
Yes	35	**2.56 (1.34-4.92)**	53	**4.57 (2.27-9.18)**
Myometrial invasion				
≤1/2 myometrial	36	1.00 (reference)	172	1.00 (reference)
>1/2 myometrial	39	1.76 (0.92-3.37)	111	**2.42 (1.22-4.78)**
Maximum diameter tumor	63	1.13 (0.95-1.35)	103	**1.05 (1.01-1.10)**
Cervical cytology				
Normal	10	1.00 (reference)	180	1.00 (reference)
Abnormal	66	1.73 (0.61-4.89)	103	1.41 (0.72-2.77)

[*]Not available: the variable 'tumor grade' is a constant in UPSC patients. [$]Not available: the variable 'histology' is a constant in EEC patients. LVSI: lymphovascular space invasion; UPSC: uterine papillary serous carcinoma; EEC: endometrioid endometrial carcinoma. [#]NA: Not available because the number of EEC patients with proper lymph node sampling was insufficient.

In EEC patients abnormal cervical cytology was significantly associated with cervical involvement (*P*=0.034). All other clinicopathologic variables were not associated with abnormal cervical cytology in EEC patients (Table 3). Cervical involvement was the only variable associated with abnormal cervical cytology in EEC patients using univariable logistic regression (OR 1.61, 95% CI 1.14–4.88; data not shown).

Survival analysis

In a Cox proportional hazard model clinicopathologic variables including preoperative cervical cytology were analyzed for their association with progression free survival (PFS). In univariable analyses in UPSC patients, FIGO stage, extra-uterine disease, lymph node metastases, and LVSI were significantly associated with PFS (Table 4). Abnormal cervical cytology was not associated with PFS in UPSC patients. In addition, no associations were found between PFS and age, BMI, cervical involvement, myometrial invasion, tumor diameter, or the composition of the tumor (pure or mixed UPSC). In EEC patients, FIGO stage, tumor grade, extra-uterine disease, cervical involvement, LVSI, myometrial invasion, and tumor diameter were significantly associated with PFS (Table 4). However, also in EEC patients abnormal cervical cytology specific for endometrial pathology was not associated with PFS.

Discussion

In this study a high frequency of abnormal cervical cytology was found in UPSC patients compared to EEC patients (87.5% and 37.8% respectively). In UPSC patients, abnormal cervical cytology was significantly associated with extra-uterine disease, whereas in EEC patients an association was found with cervical involvement. Abnormal cervical cytology specific for endometrial pathology was not associated with survival in either UPSC or EEC patients.

The possible prognostic and diagnostic role of cervical cytology in patients with the suspicion of endometrial cancer has gained little attention so far. It was shown that cervical cytology may provide additional preoperative diagnostic information when normal, suspicious or malignant endometrial cells are detected. The presence of atypical endometrial cells has a significant correlation with the presence of endometrial cancer.[15,17,18,39] In addition,

in endometrial cancer patients abnormal cervical cytology has been associated with unfavorable prognostic, clinical, and pathologic parameters.[19,20,22,24,25,38,40] However, in none of these studies a difference was made between patients with EEC and UPSC.

The most striking finding in this study was the very high frequency of abnormal cervical cytology (87.5%) in UPSC patients relative to EEC patients. At present, there have only been a few studies with a limited number of patients on cervical cytology in UPSC patients, and frequencies of abnormal cervical cytology among UPSC patients have varied from 72-88%.[25,27-30] It has been suggested previously that cervical cytology of UPSC patients is more likely to contain suspicious or malignant endometrial cells, probably due to the papillary architecture of the tumor and the propensity to exfoliate.[38,41] Although the molecular biology to account for this observation has not been thoroughly investigated, we and others propose there is a relation with change of expression of cell adhesion molecules such as CD44, integrin, E-cadherin, β-catenin and L1CAM.[40-42] In addition, involvement of the endocervix by the serous uterine tumor is more prevalent when compared to EEC and hence could explain the high rate of positive cervical cytology.[41,43]

We showed that in UPSC patients extra-uterine disease was significantly associated with abnormal cervical cytology. Markedly, UPSC patients who lacked poor prognostic factors still had abnormal cervical cytology in most cases. This was illustrated by patients with negative peritoneal cytology or absence of lymph node metastases who had abnormal cervical cytology in 92.5% and 95.2% of the cases respectively. In EEC patients, we found an association of abnormal cervical cytology with cervical involvement. The prognostic impact of other clinicopathologic factors was in concordance with literature.[1,17] We did not find a prognostic impact of BMI in our analyses, probably due to our separate analyses of the EEC and UPSC cohort. Furthermore, the number of EEC patients might not be sufficient to find a prognostic impact of BMI.

Investigators have studied whether preoperative cervical cytology is an independent prognostic factor for survival in endometrial cancer patients. Although in one study by Fukuda *et al* an association was found in univariable analyses,[24] cervical cytology never was an independent prognosticator for survival. In concordance, we found no association between cervical cytology and progression free survival in both UPSC and EEC patients independently. The fact that abnormal cervical cytology was associated with extra-uterine

disease but not with PFS in UPSC patients may be explained by the very large number of UPSC patients with advanced stage of disease with a dismal prognosis.

It was shown by some investigators that preoperative cervical cytology with atypical glandular/ endocervical cells, especially in patients over 50 years of age, was associated with endometrial pathology in 5-25% of cases.[44,45] In our UPSC cohort two patients had atypical glandular cells, and in the EEC cohort one patient had atypical glandular cells. When these smears were considered as abnormal cytology, results of the analyses were not different. In the UPSC group extra-uterine spread of disease remained the only variable significantly associated with an increased frequency of abnormal cervical cytology (OR 5.33, 95% CI 1.12 – 29.54; data not shown), whereas cervical involvement remained the only variable significantly associated with abnormal cervical cytology in EEC patients (OR 5.67 95% CI 1.07-30.09; data not shown). Furthermore, abnormal cervical cytology was still not associated with PFS in both cohorts (data not shown).

It is of great importance to identify UPSC already in the preoperative setting, because of UPSC's aggressive behavior and different surgical and adjuvant treatment approach compared to its EEC counterpart, including radical debulking surgery, comprehensive staging and chemoradiation therapy.[14,46] Biopsy of the endometrium using either outpatient biopsy techniques or dilatation and curettage has been the standard of care procedure to obtain a preoperative histopathologic diagnosis.[6] However, diagnosis of the histologic type made on these biopsies or curettage specimen can be challenging, with up to 20% of the diagnoses of histologic type being changed after surgery.[47] Preoperative cervical cytology might give an indication to suspect a more aggressive uterine tumor.

There are some limitations to this study, being retrospective as most important issue. In only a small portion of included EEC patients lymphadenectomy or lymph node sampling was performed, and not all UPSC patients were radically debulked and comprehensively staged for reasons like massive spread of disease, morbid obesity or medical co-morbidities. In addition, there was a change from conventional to liquid based cytology at different time points in the different laboratories involved. The sensitivity for the detection of endometrial cancer appears to be higher when liquid based cytology is used compared to conventional techniques.[48] However, we cannot comment on the difference in detection rate in the current study, since data on the cytology method are not available for all individual patients. Furthermore, we found a difference in median time of follow up between the EEC and

UPSC cohort. However, this difference can be explained by the highly aggressive nature of UPSC, with its high mortality rate already soon after clinical presentation, and poor five-year overall survival rate compared to EEC patients. This multicentre study comprises patients of five different institutions, with UPSC and EEC histology confirmed by three dedicated gynecopathologists, and with a complete national coverage of cervical cytology history. Furthermore, in all included patients cervical cytology was taken before endometrial biopsy or dilatation & curettage, with a median time interval of 1-2 months prior to final histopathologic diagnosis. To our knowledge this is the first study analyzing endometrial pathology in cervical cytology in two large cohorts of UPSC and EEC patients specifically.

In conclusion, abnormal cervical cytology was more frequently found in UPSC patients. It was associated with extra-uterine disease in UPSC patients and with cervical involvement in EEC patients. More prospective research should be performed to assess the true clinical value of preoperative cervical cytology in endometrial cancer patients.

References

1. Amant F, Moerman P, Neven P, et al. Endometrial cancer. *Lancet* 2005;366:491-505.
2. Bokhman JV. Two pathogenetic types of endometrial carcinoma. *Gynecol Oncol* 1983;15:10-7.
3. Ronnett BM, Zaino RJ, Hedrick Ellenson L, Kurman RJ. Endometrial carcinoma. In: Kurman R.J, TeLinde R.W, editors. Blaustein's Pathology of the Female Genital tract. 5 ed. Springer; 2000 p. 501-46.
4. Hamilton CA, Kapp DS, Chan JK. Clinical aspects of uterine papillary serous carcinoma. *Curr Opin Obstet Gynecol* 2008;20:26-33.
5. Grice J, Ek M, Greer B et al. Uterine papillary serous carcinoma: evaluation of long-term survival in surgically staged patients. *Gynecol Oncol* 1998;69:69-73.
6. Mariani A, El-Nashar SA, Dowdy SC. Lymphadenectomy in endometrial cancer: which is the right question? *Int J Gynecol Cancer* 2010;20:S52-4.
7. Kitchener H, Swart AM, Qian Q, et al. Efficacy of systematic pelvic lymphadenectomy in endometrial cancer (MRC ASTEC trial): a randomised study. *Lancet* 2009;373:125-36.
8. Ambros RA, Sherman ME, Zahn CM, et al. Endometrial intraepithelial carcinoma: a distinctive lesion specifically associated with tumors displaying serous differentiation. *Hum Pathol* 1995;26:1260-7.
9. Sturgeon SR, Sherman ME, Kurman RJ et al. Analysis of histopathological features of endometrioid uterine carcinomas and epidemiologic risk factors. *Cancer Epidemiol Biomarkers Prev* 1998;7:231-5.
10. Gehrig PA, Groben PA, Fowler WC, Jr., et al. Noninvasive papillary serous carcinoma of the endometrium. *Obstet Gynecol* 2001;97:153-7.
11. Geisler JP, Geisler HE, Melton ME, et al. What staging surgery should be performed on patients with uterine papillary serous carcinoma? *Gynecol Oncol* 1999;74:465-7.
12. Bristow RE, Asrari F, Trimble EL, et al. Extended surgical staging for uterine papillary serous carcinoma: survival outcome of locoregional (Stage I-III) disease. *Gynecol Oncol* 2001;81:279-86.
13. Chan JK, Loizzi V, Youssef M et al. Significance of comprehensive surgical staging in noninvasive papillary serous carcinoma of the endometrium. *Gynecol Oncol* 2003;90:181-5.
14. Schwartz PE. The management of serous papillary uterine cancer. *Curr Opin Oncol* 2006;18:494-9.
15. Zucker PK, Kasdon EJ, Feldstein ML. The validity of Pap smear parameters as predictors of endometrial pathology in menopausal women. *Cancer* 1985; 56:2256-63.
16. Mitchell H, Giles G, Medley G. Accuracy and survival benefit of cytological prediction of endometrial carcinoma on routine cervical smears. *Int J Gynecol Pathol* 1993;12:34-40.
17. Siebers AG, Verbeek AL, Massuger LF, et al. Normal appearing endometrial cells in cervical smears of asymptomatic postmenopausal women have predictive value for significant endometrial pathology. *Int J Gynecol Cancer* 2006;16:1069-74.
18. Yancey M, Magelssen D, Demaurez A, et al. Classification of endometrial cells on cervical cytology. *Obstet Gynecol* 1990;76:1000-5.
19. Dubeshter B, Deuel C, Gillis S, et al. Endometrial cancer: the potential role of cervical cytology in current surgical staging. *Obstet Gynecol* 2003;101:445-50.

20. Morimura Y, Nishiyama H, Hashimoto T et al. Diagnosing endometrial carcinoma with cervical involvement by cervical cytology. *Acta Cytol* 2002; 46(2):284-290.
21. Zuna RE, Erroll M. Utility of the cervical cytologic smear in assessing endocervical involvement by endometrial carcinoma. *Acta Cytol* 1996;40:878-84.
22. Dubeshter B, Warshal DP, Angel C, et al. Endometrial carcinoma: the relevance of cervical cytology. *Obstet Gynecol* 1991;77:458-62.
23. Dubeshter B. Endometrial cancer: predictive value of cervical cytology. *Gynecol Oncol* 1999;72:271-2.
24. Fukuda K, Mori M, Uchiyama M et al. Preoperative cervical cytology in endometrial carcinoma and its clinicopathologic relevance. *Gynecol Oncol* 1999;72:273-7.
25. Larson DM, Johnson KK, Reyes CN, Jr., et al. Prognostic significance of malignant cervical cytology in patients with endometrial cancer. *Obstet Gynecol* 1994;84:399-403.
26. Brown AK, Gillis S, Deuel C, et al. Abnormal cervical cytology: a risk factor for endometrial cancer recurrence. *Int J Gynecol Cancer* 2005;15:517-22.
27. Kuebler DL, Nikrui N, Bell DA. Cytologic features of endometrial papillary serous carcinoma. *Acta Cytol* 1989;33:120-6.
28. Park JY, Kim HS, Hong SR, et al. Cytologic findings of cervicovaginal smears in women with uterine papillary serous carcinoma. *J Korean Med Sci* 2005;20:93-7.
29. Todo Y, Minobe S, Okamoto K et al. Cytological features of cervical smears in serous adenocarcinoma of the endometrium. *Jpn J Clin Oncol* 2003;33:636-41.
30. Skaznik-Wikiel ME, Ueda SM, Frasure HE et al. Abnormal cervical cytology in the diagnosis of uterine papillary serous carcinoma: earlier detection of a poor prognostic cancer subtype? *Acta Cytol* 2011;55:255-60.
31. Casparie M, Tiebosch AT, Burger G et al. Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. *Cell Oncol* 2007;29:19-24.
32. Hendrickson M, Ross J, Eifel P, Martinez A, Kempson R. Uterine papillary serous carcinoma: a highly malignant form of endometrial adenocarcinoma. *Am J Surg Pathol* 1982;6:93-108.
33. Sherman ME, Bitterman P, Rosenshein NB, Delgado G, Kurman RJ. Uterine serous carcinoma. A morphologically diverse neoplasm with unifying clinicopathologic features. *Am J Surg Pathol* 1992;16:600-10.
34. Fader AN, Starks D, Gehrig PA, et al. An updated clinicopathologic study of early-stage uterine papillary serous carcinoma (UPSC). *Gynecol Oncol* 2009;115:244-8.
35. Siebers AG, Klinkhamer PJ, Arbyn M, et al. Cytologic detection of cervical abnormalities using liquid-based compared with conventional cytology: a randomized controlled trial. *Obstet Gynecol* 2008;112:1327-34.
36. Hanselaar AG. Criteria for organized cervical screening programs. Special emphasis on The Netherlands program. *Acta Cytol* 2002;46:619-29.
37. Creasman WT. New gynecologic cancer staging. *Obstet Gynecol* 1990;75:287-8.
38. Gu M, Shi W, Barakat RR, et al. Pap smears in women with endometrial carcinoma. *Acta Cytol* 2001;45:555-60.

39. Van den Bosch T, Vandendael A, Wranz PA, et al. Cervical cytology in menopausal women at high risk for endometrial disease. *Eur J Cancer Prev* 1998;7:149-52.
40. Brown AK, Gillis S, Deuel C, et al. Abnormal cervical cytology: a risk factor for endometrial cancer recurrence. *Int J Gynecol Cancer* 2005;15:517-22.
41. Lozowski MS, Mishriki Y, Solitare GB. Factors determining the degree of endometrial exfoliation and their diagnostic implications in endometrial adenocarcinoma. *Acta Cytol* 1986;30:623-7.
42. Huszar M, Pfeifer M, Schirmer U et al. Up-regulation of L1CAM is linked to loss of hormone receptors and E-cadherin in aggressive subtypes of endometrial carcinomas. *J Pathol* 2010;220:551-61.
43. Schneider ML, Wortmann M, Weigel A. Influence of the histologic and cytologic grade and the clinical and postsurgical stage on the rate of endometrial carcinoma detection by cervical cytology. *Acta Cytol* 1986;30:616-22.
44. Schnatz PF, Guile M, O'Sullivan DM, Sorosky JI. Clinical significance of atypical glandular cells on cervical cytology. *Obstet Gynecol* 2006;107:701-8.
45. Lai CR, Hsu CY, Tsay SH, Li AF. Clinical significance of atypical glandular cells by the 2001 Bethesda System in cytohistologic correlation. *Acta Cytol* 2008;52:563-7.
46. Roelofsen T, van Ham MA, de Hullu JA, et al. Clinical management of uterine papillary serous carcinoma. *Expert Rev Anticancer Ther* 2011;11:71-81.
47. Huang GS, Gebb JS, Einstein MH, et al. Accuracy of preoperative endometrial sampling for the detection of high-grade endometrial tumors. *Am J Obstet Gynecol* 2007;196:243-5.
48. Sams SB, Currens HS, Raab SS. Liquid-based Papanicolaou tests in endometrial carcinoma diagnosis. Performance, error root cause analysis, and quality improvement. *Am J Clin Pathol* 2012;137:248-54.

CHAPTER 4

ABSOLUTE DEPTH OF MYOMETRIAL INVASION IN ENDOMETRIAL CANCER IS SUPERIOR TO THE CURRENTLY USED CUT-OFF VALUE OF 50%

Y.P. Geels[1], J.M.A. Pijnenborg[2], S.H.M. van den Berg-van Erp[3], M.P.M.L. Snijders[4], J. Bulten[5], L.F.A.G. Massuger[1]

1.Department of Obstetrics and Gynecology, Radboud University Nijmegen Medical Centre, The Netherlands
2. Department of Obstetrics and Gynecology, TweeSteden Hospital, Tilburg, The Netherlands
3. Department of Pathology, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands
4. Department of Obstetrics and Gynecology, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands
5. Department of Pathology, Radboud University Nijmegen Medical Centre, The Netherlands

Gynecol Oncol. 2013 May;129(2):285-91

Abstract

Objective: In endometrial carcinoma, myometrial invasion is a well-known predictor of recurrence, and important in the decision making for adjuvant treatment. According to the FIGO staging system, myometrial invasion is expressed as invasion of < 50% > of the myometrium (50%MI). It has been suggested to use the absolute depth of invasion (DOI), or the tumor free distance to the serosa (TFD). The aim of this study was to compare DOI, 50%MI, and TFD.

Methods: All patients diagnosed with endometrioid endometrial carcinoma at the RUNMC, and the CWH from 1999-2009 were included. Histologic slides were reviewed for histologic type and grade, DOI, 50%MI, and TFD. After review, 335 patients were identified. DOI, 50%MI, and TFD were evaluated for their prediction of clinicopathologic characteristics.

Results: The prediction of recurrence was best performed by DOI when compared to TFD, with an area under the ROC curve of 0.726, and 0.638 respectively. The optimal cut-off value for DOI was 4mm. DOI independently correlated with recurrence of disease, and death of disease. TFD was associated with advanced age and large tumor diameter. DOI was the best predictor of progression-free and disease-specific survival next to 50%MI and TFD (HR 3.15, 95%CI 1.16-8.56) and (HR 10.35, 95%CI 1.23-86.93).

Conclusion: DOI showed better predictive performance than TFD, and was more strongly correlated with clinicopathologic parameters than TFD and 50%MI. Possibly, DOI should substitute 50%MI as measure to express myometrial invasion in daily clinical practice. External validation is mandatory to confirm the proposed cut-off value of 4mm.

Introduction

Endometrial cancer is the most common gynecologic malignancy in western countries. The majority of patients have endometrioid endometrial carcinomas, which in general have a favorable prognosis and present in early stage disease. However, about 20% of the patients have a more aggressive type of carcinoma, and are more likely to develop recurrent disease.[1,2]

From literature, several parameters are known to predict the chance of recurrence. Age, histologic subtype, tumor grade, FIGO stage, and myometrial invasion are the most important predictors of overall and recurrence free survival.[3-6] The depth of myometrial invasion is routinely determined in patients surgically treated for endometrial carcinoma. In the FIGO staging system, the assessment whether myometrial invasion is less or more than 50% is important: it makes the difference between FIGO stage IA and IB.[7] Moreover, the depth of myometrial invasion is, together with age and tumor grade, a key parameter in the decisional process around the adjuvant radiotherapeutic treatment of endometrial carcinoma patients.[4]

However, determination of myometrial invasion may be challenging due to an irregular endomyometrial junction, exophytic tumor growth, adenomyosis, extensive leiomyomas, and different patterns of myometrial invasion.[8,9] In literature, the rate of discrepancy when myometrial invasion is reassessed is around 30%.[9,10] Incorrect assessment of myometrial invasion can lead to suboptimal staging, and hence, to suboptimal treatment. It has been suggested to use absolute depth of invasion and tumor free distance to the serosa as an alternative measurement of myometrial invasion. These measurements possibly better predict the chance of recurrent disease.[11-15]

The aim of the current study is to assess the predictive value of absolute depth of invasion and tumor free distance to the serosa, and to evaluate the relationship of these parameters with clinicopathologic factors and progression free survival. Absolute depth of myometrial invasion and tumor free distance were compared to the conventional rendering of myometrial invasion in more or less than 50% of the total myometrial thickness.

Methods

Patient selection

The Dutch nationwide network and registry of histology and cytopathology (Pathologisch Anatomisch Landelijk Geautomatiseerd Archief: PALGA) was used to search for all patients diagnosed, and surgically treated with at least hysterectomy and bilateral oophorectomy at the Radboud University Nijmegen Medical Center (RUNMC), and the Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands, for primary endometrioid endometrial carcinoma between January 1999 and January 2010. A total of 354 endometrioid endometrial carcinoma patients were identified. Clinical data were retrospectively collected by studying the medical charts. Age, menopausal state, body mass index (BMI), parity, personal medical history, (adjuvant) treatment, stage of disease, date of recurrence, date of death, and the cause of death were registered. Stage of disease was based on the 2009 International Federation of Gynecology and Obstetrics (FIGO) staging system.[7] Surgical treatment was performed according to the Dutch guidelines: In case of a pre-operative apparently stage I disease and grade 1 or 2 endometrioid endometrial carcinoma, pelvic and/or para-aortic lymphadenectomy is not recommended. In case of grade 3 endometrioid endometrial carcinoma, pelvic and para-aortic lymphadenectomy is considered.[16] Adjuvant radiotherapy was recommended to patients according to the PORTEC criteria, i.e. the presence of two out of the following three criteria: age over 60, tumor grade 3, and more than 50% myometrial invasion.[4] One patient was excluded because of a synchronous primary malignancy of the ovary.

Review of pathologic specimens

The slides of the primary endometrial carcinoma of all 353 patients were retrieved from the pathology archives and used for review. Review was done systematically including the following items: histologic type, tumor grade, myometrial thickness, depth of myometrial invasion (DOI), tumor free distance to the serosa (TFD), myometrial invasion more or less than 50% (50%MI), and the presence of lymphovascular space invasion.[17] Myometrial thickness was measured from the endomyometrial junction to the serosa in the section where the tumor demonstrated the deepest invasion. DOI was measured from the endomyometrial junction to the deepest point of myometrial invasion. The endomyometrial junction refers

to the transition from endometrium to myometrium. In case of an irregular endomyometrial junction, we determined the endomyometrial junction in the area where the tumor reached the deepest invasion. TFD was measured from the deepest point of myometrial invasion to the serosa. All measurements were performed in the same section where the tumor reached the deepest point of invasion. In case of a leiomyoma in the uterine wall in this section, the myometrial thickness in the area directly next to the leiomyoma was measured. Carcinoma involving adenomyosis was not measured as myometrial invasion. In case of carcinoma in adenomyosis, there are often some normal endometrial tubes present, and there is a sharp boundary between endometrium and myometrium.[18] A median number of three slides (range 1-8) with tumor and adjacent myometrium with serosa were available for each patient to re-evaluate the depth of myometrial invasion. Figure 1 shows examples of how measurements were performed. Review was performed in both hospitals separately by an experienced pathologist (JB, SB), on the tissue of the patients of the concerning hospital, who was unaware of the results of the original pathology reports, and the clinical outcome of the patients. A second pathologist of the concerning hospital was consulted in case of doubt about the myometrial invasion by the reviewer, or in case of discrepancy

Figure 1: Depth Of myometrial Invasion DOI, Tumor Free Distance to the serosa (TFD), endomyometrial junction (EMJ), Deepest Invasion (DI).

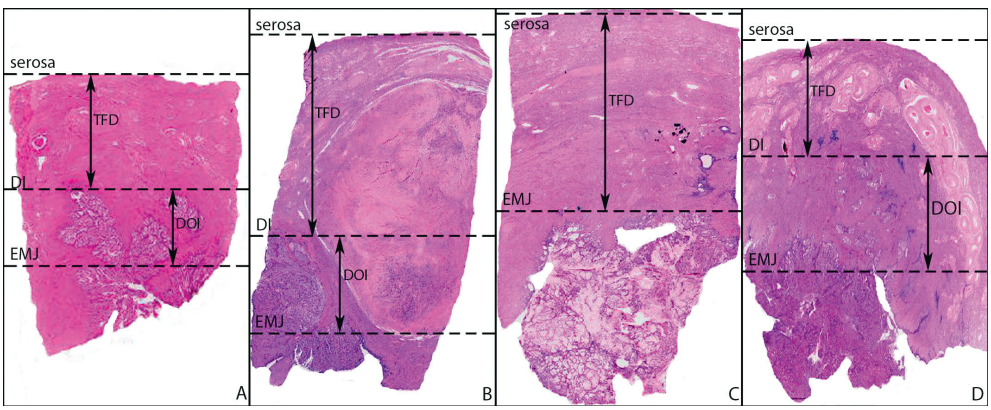


Figure 1A: Example of assessment of DOI and TFD
Figure 1B: Example of assessment of DOI and TFD in a patient with leiomyoma
Figure 1C: Example of assessment of DOI and TFD in a patient with exophytic tumor growth, there is no DOI in this case
Figure 1D: Example of assessment of DOI and TFD in a patient with deep myometrial invasion

with the initial diagnosis, and consensus about the diagnosis was achieved. After review, 18 patients were diagnosed with (partly) non-endometrioid histology and were excluded, 335 endometrioid endometrial carcinoma patients remained for analysis. A tumor was diagnosed as (partly) non-endometrioid when 10% or more of the tumor consisted of non-endometrioid histology like serous, or clear cell type.[18] The assessment whether myometrial invasion was less or more than 50% changed in 75 of the 335 patients (22%) after review (data shown in Supplemental Material 1). To note, for the results and the analyses, the reviewed pathology results were used.

Statistical analysis

Receiver operating characteristics (ROC) analyses were established to evaluate the diagnostic performance of DOI and TFD for the prediction of recurrent disease. The performance of the tests is summarized by the area under the ROC curve (AUC). By using the ROC analysis cut-off values for the best sensitivity and specificity of DOI and TFD were established. These cut-off values were used for further analysis to achieve an equal comparison with 50%MI. The correlations of DOI, TFD, and the conventional 50%MI with clinical and pathologic factors in all endometrioid endometrial carcinoma patients were made using univariable logistic regression. In addition, for the correlation with each clinicopathologic outcome measure separately, a multivariable logistic regression model was comprised entering only DOI, 50%MI, and TFD using the forward stepwise method. Results of correlation analyses were expressed in Odds Ratios (OR) with 95% Confidence Intervals (95%CI). Survival techniques were used to study the progression free survival (PFS) and disease specific survival (DSS). PFS was calculated from the date of surgery until the date of recurrence. In case of no recurrence, the date of last contact or death was used for censoring. DSS was calculated from the date of surgery till the date of death as a consequence of the endometrial carcinoma. In case of death by other cause, data were censored. The prognostic impact of variables age, BMI, FIGO stage, 50%MI, DOI, TFD, lymph node involvement, cervical involvement, tumor grade, lymphovascular space invasion, and diameter of the tumor were analyzed by using univariable and multivariable Cox proportional hazards models. The forward stepwise method was used for selection procedures for multivariable Cox proportional hazards

models. These results were expressed as Hazard Ratio's (HR) with 95%CI. Statistical analyses were performed using the software package SPSS 18.0 for Microsoft Windows (SPSS Inc).

Ethical committee approval

The Research Ethics Committee of the Radboud University Nijmegen Medical Centre declared that the study protocol is in accordance with the applicable rules concerning the review of research ethics committees and informed consent.

Results

Patient characteristics

Clinical and pathologic characteristics of all patients are shown in Table 1. A total of 335 consecutive endometrioid endometrial carcinoma patients were included in the study. As expected, the majority was diagnosed with FIGO stage I. In 37.6% of the patients myometrial invasion was more than 50% of the myometrium. Median myometrial thickness was 13.0 mm (range 3.0-30.0), median DOI was 4.0 mm (range 0-27.0), and median TFD was 7.0 mm (range 0-28.0). One-hundred-and-twenty-seven patients (38.2%) received adjuvant therapy. Median time of follow up was 47 months (range 1-128). Forty-three patients (12.8%) developed recurrent disease, and 23 patients (6.9%) died as a consequence of the endometrial carcinoma. Lymph node dissection was omitted in 279 cases with tumor grade 1 or 2 endometrioid endometrial carcinoma diagnosed on endometrial biopsy, without clinical suspicion of FIGO stage II or more, as recommended by the Dutch guidelines for endometrial cancer treatment.[15] In the eight patients diagnosed as FIGO stage IIIC1 or IIIC2 a median number of nine (range 1-29) lymph nodes were removed.

ROC curves

DOI showed a larger area under the ROC curve (AUC) than TFD for prediction of recurrence of disease: 0.726, and 0.638 respectively (Figure 2). The optimal cut off value for DOI was 3.75 mm, providing 85% sensitivity and 50% specificity. For TFD the optimal cut-off value was 7.25 mm with 74% sensitivity and 51% specificity. We further analyzed the accuracy of these cut-off values by dividing the population in patients with < 4 mm DOI and patients with ≥ 4 mm DOI. For the TFD we divided the population in patients with ≤ 7 mm TFD > and 7

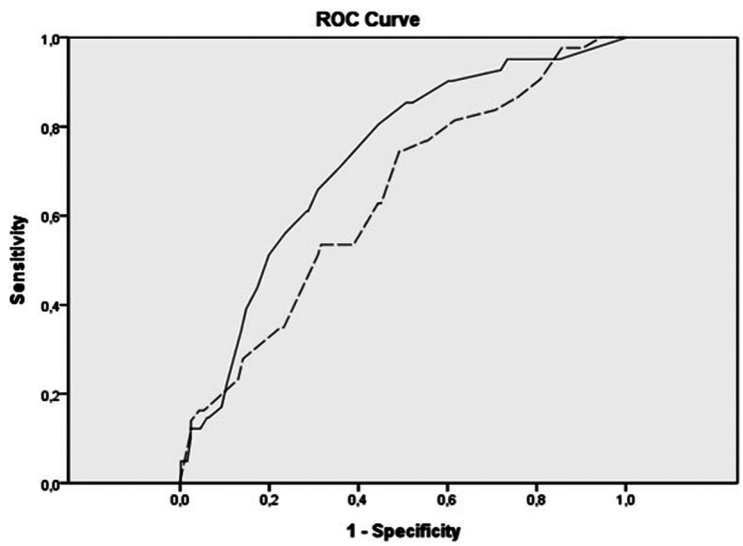
Table 1: Clinical and pathologic characteristics of all patients (N=335).

Variable	N /median	%/ range
Median age (years)	64	24-93
Median BMI* (kg/m^2)	28.1	18.4-65.0
Diabetes Mellitus		
No	281	83.9
Yes	48	14.3
Unknown	6	1.8
FIGO stage (2009)		
IA	195	58.2
IB	92	27.4
II	13	3.9
IIIA	6	1.8
IIIB	6	1.8
IIIC1	4	1.2
IIIC2	4	1.2
IVA	3	0.9
IVB	12	3.6
Myometrial Invasion		
No	47	14.0
<1/2	154	46.0
≥1/2	126	37.6
Trough serosa	8	2.4
Myometrial Thickness (mm)	13.0	3.0-30.0
Depth Of myometrial Invasion (mm)	4.0 7.0	0.0-27.0
Tumor Free Distance to serosa (mm)		0.0-28.0
Nodal involvement		
No	46	13.7
Yes	10	3.0
Unknown	279	83.3
Cervical involvement		
No	288	86.0
Endocervical	20	6.0
Stromal	27	8.0
Tumor grade		
1	142	42.4
2	136	40.6
3	57	17.0
Lymphovascular space invasion		
No	267	79.7
Yes	68	20.3
Diameter of the tumor	25.0	1.0-80.0
Time of follow up in months	47.0	0-128

Adjuvant therapy		
No adjuvant therapy	206	61.5
Radiotherapy	118	35.2
Radiotherapy (+Chemotherapy/	10	3.0
Hormonal therapy)		0.3
Unknown	1	
Recurrence		
No	286	85.3
Yes, local	19	5.6
Yes, distance	25	7.4
Unknown	6	1.7
Patient died		
No	296	88.3
Yes, as a consequence of disease	23	6.9
Yes, other cause	6	1.8
Unknown	10	3.0

*Body Mass Index

Figure 2: ROC curve of Depth of myometrial Invasion and Tumor Free Distance to the serosa for predicting recurrence of disease.



— DOI: AUC 0.726 (95%CI 0.647-0.804, *P*<0.001)
--- TFD: AUC 0.638 (95%CI 0.551-0.725, *P*=0.004)

mm TFD. By using dichotomous values for DOI and TFD, the results could be compared with the conventional 50%MI in correlation analyses with clinicopathologic parameters.

Correlation of 50%MI, DOI, and TFD with clinical and pathologic parameters

The results of correlation analyses are shown in Table 2. In univariable analysis, DOI, 50%MI and TFD were all associated with the presence of several prognostic unfavorable parameters: advanced FIGO stage, cervical involvement, poor differentiation, and lymphovascular space

Table 2: Correlation of DOI[#] using the cut-off value of <4 mm or ≥4mm, MI[$] <50% or ≥50%, and TFD[†] of ≤7mm or >7mm with clinical and pathologic factors using univariable and multivariable logistic regression.

		Univariable		Multivariable	
Outcome	Predictor	OR	95% CI	OR	95% CI
FIGO stage (2009) II, III, IV	DOI ≥ 4mm	**10.85**	**3.78-30.98**	**8.30**	**2.86-24.05**
	MI ≥ 50%	**6.84**	**3.35-14.00**	**3.98**	**1.75-9.02**
	TFD ≤ 7mm	**5.46**	**2.46-12.09**	NS	
Nodal involvement	DOI ≥ 4mm	NA[£]		NA	
	MI ≥ 50%	3.67	0.70-19.17	NA	
	TFD ≤ 7mm	7.20	0.84-61.69	NA	
Cervical involvement	DOI ≥ 4mm	**4.54**	**2.05-10.07**	**3.61**	**1.60-8.15**
	MI ≥ 50%	**3.11**	**1.63-5.90**	NS	
	TFD ≤7mm	**2.85**	**1.42-5.74**	**2.19**	**1.07-4.51**
Tumor grade 3	DOI ≥ 4mm	**2.94**	**1.51-5.72**	NS	
	MI ≥ 50%	**4.17**	**2.27-7.69**	**2.30**	**1.12-4.73**
	TFD ≤7mm	**4.55**	**2.25-9.17**	**2.76**	**1.22-6.24**
Lymphovascular space invasion	DOI ≥ 4mm	**11.22**	**4.69-26.84**	**8.61**	**3.55-20.88**
	MI ≥ 50%	**4.63**	**2.61-8.23**	NS	
	TFD ≤ 7mm	**4.28**	**2.26-8.10**	**3.03**	**1.55-5.92**
Recurrence of disease	DOI ≥ 4mm	**5.79**	**2.37-14.14**	**3.35**	**1.17-9.62**
	MI ≥ 50%	**4.29**	**2.14-8.60**	NS	
	TFD ≤ 7mm	**2.97**	**1.44-6.14**	NS	
Death as consequence of disease	DOI ≥ 4mm	**19.54**	**2.60-146.81**	**19.18**	**2.55-144.17**
	MI ≥ 50%	**6.10**	**2.20-16.86**	NS	
	TFD ≤ 7mm	**2.61**	**1.00-6.841**	NS	
Age >60 years	DOI ≥ 4mm	1.33	0.85-2.08	NS	
	MI ≥ 50%	**2.58**	**1.60-4.17**	NS	
	TFD ≤7mm	**2.93**	**1.83-4.67**	**2.86**	**1.79-4.58**
Diameter of the tumor > 20 mm	DOI ≥ 4mm	1.43	0.39-2.97	NS	
	MI ≥ 50%	**2.92**	**1.35-6.33**	NS	
	TFD ≤ 7mm	**2.84**	**1.36-5.96**	**2.69**	**1.28-5.67**

[#]Depth Of myometrial Invasion, [$]Myometrial Invasion, [†]Tumor Free Distance to serosa, *Body Mass Index, [£]Not Applicable, [&]Not Selected

invasion. Furthermore, all three methods of defining myometrial invasion were associated with recurrent disease, and death as a consequence of disease. Differences in correlation of DOI, 50%MI, and TFD with clinicopathologic parameters were found for age, and tumor diameter. Age ≥ 60 years and tumor diameter ≥ 20 mm were associated with TFD and 50%MI, but not with DOI. Note that Odd's Ratios of DOI were higher when compared to 50%MI and TFD in almost all analyses, except for tumor grade, age, and tumor diameter. In multivariable analysis for each clinicopathologic outcome measure separately, recurrent disease, and death as a consequence of disease. Furthermore, DOI was most strongly associated with advanced FIGO stage, cervical involvement, and lymphovascular space invasion. TFD was independently associated with advanced age and large tumor diameter. TFD and 50%MI were equally correlated with high tumor grade.

Survival analysis

In univariable Cox proportional hazard's models (Table 3) advanced FIGO stage, ≥ 50 %MI, ≥ 4 mm DOI, ≤ 7 mm TFD, cervical involvement, high tumor grade, and the presence of lymphovascular space invasion were all significant predictors of progression free survival and disease specific survival. In addition, tumor diameter was a significant predictor of disease specific survival. In a multivariable Cox proportional hazard's model (Table 4) only FIGO stage and tumor grade remained as predictors of poor progression free survival. In the multivariable model for disease specific survival FIGO stage, tumor grade, and DOI remained as significant predictors. Table 5 shows univariable Cox proportional hazard's models in the group with only FIGO stage I patients (N=287). In this group DOI, 50%MI, tumor grade and the presence of lymphovascular space invasion were predictors of progression free survival and disease specific survival. In a multivariable Cox proportional hazard's model in this group with FIGO stage I patients DOI and tumor grade remained as independent predictors of progression free survival and disease specific survival (Table 6). To be able to compare the three methods of defining myometrial invasion, we created a second multivariable model in which only DOI, 50%MI, and TFD were entered (Supplemental Material 2). DOI and 50%MI remained as predictors of progression free survival. DOI was found to be the strongest predictor of progression free survival with a higher hazard ratio than 50%MI. For disease specific survival DOI remained as independent predictor.

Table 3: Crude hazard ratios (HR) of progression free survival (PFS) and Disease Specific Survival (DSS) by clinicopathologic variables using univariable Cox regression.

		PFS		DSS	
Variable	N	HR	95%CI	HR	95%CI
Age (years)	328	1.02	0.99-1.05	0.98	0.94-1.03
BMI (kg/m^2)	282	0.98	0.92-1.03	1.01	0.95-1.07
FIGO stage (2009)					
I	281	1.00	reference	1.00	reference
II, III, IV	48	**8.22**	**4.54-14.89**	**8.90**	**3.90-20.22**
Depth Of myometrial Invasion					
< 4mm	143	1.00	reference	1.00	reference
≥ 4mm	182	**5.26**	**2.22-12.47**	**18.41**	**2.48-136.62**
Myometrial Invasion					
<50%	199	1.00	reference	1.00	reference
≥50%	130	**3.98**	**2.08-7.40**	**5.79**	**2.15-15.58**
Tumor Free Distance					
≤ 7mm	166	**2.77**	**1.40-5.50**	**2.55**	**1.00-6.46**
> 7mm	149	1.00	reference	1.00	reference
Nodal involvement					
No	46	1.00	reference	1.00	reference
Yes	8	2.08	0.59-7.40	0.87	0.11-7.02
Cervical involvement					
No	282	1.00	reference	1.00	reference
Yes (endocervical + stromal)	46	**3.67**	**1.94-6.96**	**4.20**	**1.76-10.02**
Tumor grade					
1	139	1.00	reference	1.00	reference
2	133	**2.47**	**1.02-6.00**	1.71	0.41-7.14
3	57	**8.93**	**3.79-21.03**	**14.92**	**4.32-56.61**
Lymphovascular space invasion					
No	262	1.00	reference	1.00	reference
Yes	66	**4.20**	**2.30-7.66**	**7.24**	**3.13-16.75**
Diameter of the tumor	213	1.03	0.99-1.07	**1.06**	**1.02-1.10**

Clinical implications of DOI instead of 50%MI

If the DOI cut-off value of ≥ 4 mm would be a criterion for adjuvant radiotherapy instead of ≥ 50%MI, 22 additional patients would fulfill the criteria for adjuvant radiotherapy. Three of these 22 patients developed recurrent disease, one with distant and two with local recurrences. In addition, using the cut-off value of ≥ 4 mm DOI would implicate omission of

Table 4: Adjusted hazard ratios (HR) of progression free survival (PFS) and Disease Specific Survival (DSS) by clinicopathologic variables using multivariable Cox regression.

		PFS		DSS	
Variable	N	HR	95%CI	HR	95%CI
FIGO stage (2009)					
I	266	1.00	reference	1.00	reference
II, III, IV	46	**5.12**	**2.65-9.89**	**2.93**	**1.16-7.36**
Depth Of myometrial Invasion					
< 4mm	NS[&]			1.00	reference
≥ 4mm				**8.43**	**1.07-66.46**
Myometrial Invasion					
< 50%	NS			NS	
≥ 50%					
Tumor Free Distance					
≤ 7mm	NS			NS	
> 7mm					
Cervical involvement					
No	NS			NS	
Yes (endocervical + stromal)					
Tumor grade					
1	132	1.00	reference	1.00	reference
2	126	1.77	0.71-4.40	0.80	0.18-3.64
3	54	**4.39**	**1.74-11.08**	**5.74**	**1.52-21.72**
Lymphovascular space invasion					
No	NS				
Yes					

[&]Not Selected

radiotherapy in five patients who had received radiotherapy based on the reviewed 50%MI. Of these five patients, one patient developed distant recurrent disease. Within the group that did not receive radiotherapy (N=188), patients who would have received radiotherapy based on the newly proposed cut-off value of 4 mm had a hazard ratio of 1.67 (95%CI 0.36-1.74) to develop recurrence of disease compared to the patients who would not have received radiotherapy based on the newly proposed cut-off value.

Table 5: Crude hazard ratios (HR) of progression free survival (PFS) and Disease Specific Survival (DSS) by clinicopathologic variables using univariable Cox regression in the group of patients with FIGO stage I disease (N=287).

		PFS		DSS	
Variable	N	HR	95%CI	HR	95%CI
Age (years)	274	1.00	0.96-1.05	0.96	0.90-1.02
BMI (kg/m^2)	236	0.92	0.84-1.02	0.92	0.80-1.05
Depth Of myometrial Invasion					
< 4 mm	139	1.00	reference	1.00	reference
≥ 4 mm	140	**3.49**	**1.83-9.45**	**10.13**	**1.30-79.11**
Myometrial Invasion					
<50%	189	1.00	reference	1.00	reference
≥ 50%	93	**2.45**	**1.06-5.68**	**3.51**	**1.03-12.00**
Tumor Free Distance					
≤ 7 mm	128	1.95	0.82-4.64	1.87	0.55-6.40
> 7 mm	141	1.00	reference	1.00	reference
Tumor grade					
1	134	1.00	reference	1.00	reference
2	113	1.35	0.49-3.72	0.74	0.13-4.46
3	35	**4.14**	**1.45-11.80**	**8.45**	**2.11-33.83**
Lymphovascular space invasion					
No	242	1.00	reference	1.00	reference
Yes	40	**2.71**	**1.06-6.94**	**6.01**	**1.83-19.73**
Diameter of the tumor	107	1.04	0.99-1.09	1.06	1.00-1.11

Table 6: Adjusted hazard ratios (HR) of progression free survival (PFS) and Disease Specific Survival (DSS) by clinicopathologic variables using multivariable Cox regression in the group of patients with FIGO stage I disease (N=287).

		PFS		DSS	
Variable	N	HR	95%CI	HR	95%CI
Depth Of myometrial Invasion					
≤ 4 mm	139	1.00	reference	1.00	reference
> 4 mm	140	**3.11**	**1.12-8.60**	**9.05**	**1.14-71.97**
Myometrial Invasion					
< 50%	188	NS[&]		NS	
≥ 50%	91				
Tumor grade					
1	134	1.00	reference	1.00	reference
2	112	1.08	0.38-3.01	0.53	0.87-3.17
3	33	**3.34**	**1.15-9.68**	**6.12**	**1.51-24.76**
Lymphovascular space invasion					
No	240	NS		NS	
Yes	39				

[&]Not Selected

Discussion

Myometrial invasion is an important predictor of recurrence and survival. In endometrioid endometrial carcinoma it is therefore a crucial parameter for the decision about adjuvant radiotherapy. In this study, the prognostic value and relationship with clinicopathologic parameters of DOI, TFD and 50%MI were analyzed and compared. DOI revealed a larger area under the ROC curve for prediction of recurrence than TFD, and the most discriminating cut-off value for DOI was 4 mm. DOI had a stronger correlation with unfavorable clinicopathologic parameters, and was a better predictor of progression free survival than TFD and 50%MI.

In endometrial carcinoma, the currently used cut-off value for adjuvant therapy is a percentage of invasion of the total myometrial thickness. In other gynecologic carcinomas absolute cut-off values are used for guidance in the choice of treatment. In vulvar carcinoma a cut-off value of 1 mm is used to decide on groin lymphadenectomy.[19] In cervical carcinoma the extent of surgery and the choice for radiotherapy are based on invasion depths of 5 mm and 15 mm, respectively.[20,21] Our results show that a cut-off value of 4mm DOI and 7 mm TFD led to the most effective balance between sensitivity and specificity. To our knowledge, for DOI no cut-off value has been suggested before. For TFD a cut-off value of 1 cm has been suggested in two reports. However, in these reports TFD was found to be only of modest predictive value.[12,13] In addition, an optimal TFD cut-off value of 1.75 mm has been reported, which showed to be a good predictor of progression free and disease specific survival.[15] In the current study, DOI was a better predictor of recurrence than TFD, showing a larger area under the ROC curve. Therefore, DOI with this absolute cut-off value of 4 mm, may be preferable in endometrial carcinoma as well.

When compared to the conventional 50%MI, the newly proposed cut-off value of 4 mm has important clinical consequences: 22 additional patients would have received adjuvant radiotherapy. More importantly, three of these 22 patients developed recurrent disease, one distant and two local recurrences. The two local recurrences may have been prevented with adjuvant radiotherapy. In future research, the clinical implications of expressing myometrial invasion as DOI should be further explored. Moreover, the cut-off value of 4 mm should be externally validated, preferably in prospective research.

Several authors have reported on optimizing the definition of myometrial invasion, with conflicting results. Lindauer *et al* reported that TFD is a better predictor of clinicopathologic

factors than DOI in a completely staged cohort of 153 patients.[12] Kaku *et al* identified TFD as independent prognostic predictor in 88 stage I and II endometrial cancer patients.[11] Chattopadhyay *et al* reported that TFD is an independent predictor of survival and lymph node metastases in 288 endometrioid endometrial carcinoma patients.[15] However, Schwab et al prospectively determined TFD, and did not find a difference in prediction of recurrence and death compared to DOI in 99 endometrial cancer patients.[13] Moreover, in the study of Kondalsamy-Chennakesavan *et al*, DOI was found to be a better predictor for the presence of lymph node metastases than TFD and %MI in 338 endometrioid endometrial carcinoma patients.[14] In three of the five above mentioned studies no review of the histologic slides was performed. Chattopadhyay *et al* reviewed slides to determine DOI and TFD.[15] Only in the study of Kaku *et al* pathologic diagnosis was confirmed with independent histologic review.[11] In the current study, DOI was a better predictor of recurrence than TFD, revealing a stronger correlation with clinicopathologic variables, and an independent correlation with recurrent disease and death as a consequence of disease when compared to TFD and 50%MI. This superior correlation could be explained by the assumption that DOI better shows the potential of the tumor to invade the myometrium.

DOI, 50%MI, and TFD were all predictors of progression free survival in univariable analyses, although in a multivariable model FIGO stage and tumor grade remained as the strongest predictors. In the multivariable model of disease specific survival DOI remained as independent predictor next to FIGO stage and tumor grade. However, the 95%CI of DOI was large, due to few deaths as a consequence of disease, making the interpretation of these results difficult. When we compared the three ways of expressing myometrial invasion, DOI was a stronger predictor of progression free survival and disease specific survival. However in this model the influences of other prognostic parameters are ignored and these results may therefore be biased. In FIGO stage I patients the correct assessment of myometrial invasion is more important to provide prognostic information. In this particular group DOI independently predicted progression free survival and disease specific survival.

Another reason for reconsidering 50%MI as a measure for myometrial invasion is the moderate reproducibility. The rate of discrepancy when 50%MI is reassessed is around 30%.[9,10] We found a similar discrepancy rate between the initial pathology report and the reviewed data of 22%. Several studies have discussed the most important factors contributing to difficulties in the assessment of myometrial invasion. The most common reasons for underestimation of

myometrial invasion are leiomyomas, whereas the most common reasons for overestimation are exophytic tumor growth, and adenomyosis. In addition, an irregular endomyometrial junction may cause discrepancies in the measurement of myometrial invasion.[9,10] For the pathologist, DOI is more practical to measure. The advantage of DOI above 50%MI is the fact that measuring myometrial thickness is not necessary. Hence, difficulties with assessing the myometrial thickness caused by leiomyomas are avoided. This is in contrast to the TFD, in which leiomyomas would cause difficulties as well. Furthermore, only one measurement is needed for DOI, for TFD and 50%MI two measurements are needed, enhancing the chance on disagreement. To our knowledge, no studies comparing the reproducibility of the three ways of assessing myometrial invasion exist yet. Currently, we are planning a study to compare the intra-observer variability of DOI, 50%MI, and TFD to confirm this hypothesis.

The shortcoming of this study is that it is a retrospective study. According to the endometrial carcinoma guidelines, a part of the study population received adjuvant radiotherapy, and a part did not. This means that the occurrence of recurrent disease is influenced by the adjuvant treatment strategy. Furthermore, in just a small portion of the study population lymphadenectomy was performed. Therefore, the prediction of the presence of lymph node metastases, and the predictive value of lymph node metastases have to be interpreted with caution. On the other hand, this study describes a large cohort, consisting only of endometrioid endometrial carcinoma patients. The exclusion of non-endometrioid histology prevents bias from results caused by significantly worse clinical behavior and prognosis of non-endometrioid tumors.[18] Furthermore, diagnosis of endometrioid histology was confirmed by reviewing all histologic slides, and DOI and TFD have been measured by experienced pathologists.

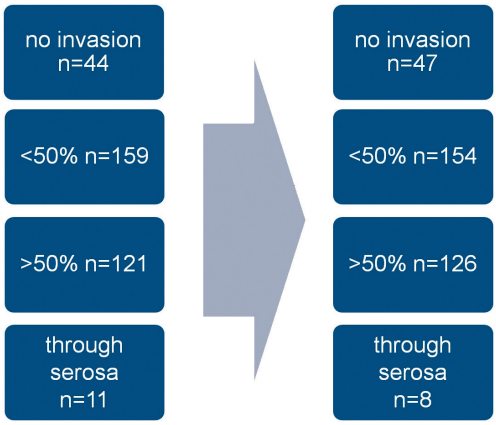
In conclusion, DOI shows superior predictive performance compared to TFD, and was more strongly correlated with clinicopathologic parameters than TFD and 50%MI. Possibly, DOI should substitute 50%MI as objective measure to express myometrial invasion in daily clinical practice. External validation is mandatory to confirm the proposed cut-off value of 4 mm as most relevant clinical criterion for myometrial invasion.

References

1. Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, Vergote I. Endometrial cancer. Lancet. 2005;366(9484):491-505. Epub 2005/08/09.
2. Geels YP, Pijnenborg JM, van den Berg- van Erp SH, Bulten J, Visscher DW, Dowdy SC, Massuger LF. Endometrioid endometrial carcinoma with atrophic endometrium and poor prognosis. Obstet Gynecol. 2012; 120(5): 1124-31. Epub 2012/10/24
3. Zaino RJ, Kurman RJ, Diana KL, Morrow CP. Pathologic models to predict outcome for women with endometrial adenocarcinoma: the importance of the distinction between surgical stage and clinical stage--a Gynecologic Oncology Group study. Cancer. 1996;77(6):1115-21. Epub 1996/03/15.
4. Creutzberg CL, van Putten WL, Koper PC, Lybeert ML, Jobsen JJ, Warlam-Rodenhuis CC, et al. Surgery and postoperative radiotherapy versus surgery alone for patients with stage-1 endometrial carcinoma: multicentre randomised trial. PORTEC Study Group. Post Operative Radiation Therapy in Endometrial Carcinoma. Lancet. 2000;355(9213):1404-11. Epub 2000/05/03.
5. Larson DM, Connor GP, Broste SK, Krawisz BR, Johnson KK. Prognostic significance of gross myometrial invasion with endometrial cancer. Obstet Gynecol. 1996;88(3):394-8. Epub 1996/09/01.
6. Prat J. Prognostic parameters of endometrial carcinoma. Hum Pathol. 2004;35(6):649-62. Epub 2004/06/10.
7. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Int J Gynaecol Obstet. 2009;105(2):103-4. Epub 2009/04/16.
8. Quick CM, May T, Horowitz NS, Nucci MR. Low-grade, low-stage endometrioid endometrial adenocarcinoma: a clinicopathologic analysis of 324 cases focusing on frequency and pattern of myoinvasion. Int J Gynecol Pathol. 2012;31(4):337-43. Epub 2012/06/02.
9. Ali A, Black D, Soslow RA. Difficulties in assessing the depth of myometrial invasion in endometrial carcinoma. Int J Gynecol Pathol. 2007;26(2):115-23. Epub 2007/04/07.
10. Jacques SM, Qureshi F, Munkarah A, Lawrence WD. Interinstitutional surgical pathology review in gynecologic oncology: II. Endometrial cancer in hysterectomy specimens. Int J Gynecol Pathol. 1998;17(1):42-5. Epub 1998/02/25.
11. Kaku T, Tsuruchi N, Tsukamoto N, Hirakawa T, Kamura T, Nakano H. Reassessment of myometrial invasion in endometrial carcinoma. Obstet Gynecol. 1994;84(6):979-82. Epub 1994/12/01.
12. Lindauer J, Fowler JM, Manolitsas TP, Copeland LJ, Eaton LA, Ramirez NC, et al. Is there a prognostic difference between depth of myometrial invasion and the tumor-free distance from the uterine serosa in endometrial cancer? Gynecol Oncol. 2003;91(3):547-51. Epub 2003/12/17.
13. Schwab KV, O'Malley DM, Fowler JM, Copeland LJ, Cohn DE. Prospective evaluation of prognostic significance of the tumor-free distance from uterine serosa in surgically staged endometrial adenocarcinoma. Gynecol Oncol. 2009;112(1):146-9. Epub 2008/10/22.
14. Kondalsamy-Chennakesavan S, van Vugt S, Sanday K, Nicklin J, Land R, Perrin L, et al. Evaluation of tumor-free distance and depth of myometrial invasion as prognostic factors for lymph node metastases in endometrial cancer. Int J Gynecol Cancer. 2010;20(7):1217-21. Epub 2011/04/19.

15. Chattopadhyay S, Galaal KA, Patel A, Fisher A, Nayar A, Cross P, et al. Tumour-free distance from serosa is a better prognostic indicator than depth of invasion and percentage myometrial invasion in endometrioid endometrial cancer. BJOG : an international journal of obstetrics and gynaecology. 2012;119(10):1162-70. Epub 2012/07/19.
16. www.oncoline.nl. 2011.
17. Silverberg SG, Mutter GL, Kurman RJ, Kubik-Huch RA, Nogales F, Tavassoli FA. Tumors of the uterine corpus: epithelial tumors and related lesions. In: avassoli FA SF, editors, editor. WHO classification of tumors: pathology and genetics of tumors of the breast and female genital organs. 5th ed: World Health Organisation; 2003. p. 221-32.
18. Ellenson HL, Ronnett BM, Soslow RA, Zaino RJ, Kurman RJ. Endometrial carcinoma. In: Kurman RJ, Ronnett BM, Ellenson HL, editors. Blaustein's Pathology of the Female Genital Tract. Sixth ed. New York: Springer; 2011. p. 393-452.
19. Wilkinson EJ. Premalignant and Malignant Tumors of the Vulva. In: Kurman RJ, Ellenson HL, Ronnett BM, editors. Blaustein's Pathology of the Female Genital Tract. sixth ed: Springer; 2011. p. 56-103.
20. Witkiewicz AK, Wright TC, RFerenczy A, Ronnett BM, Kurman RJ. Carcinoma and Other Tumors of the Cervix. In: Kurman RJ, Ellenson HL, Ronnett BM, editors. Blaustein's Pathology of the Female Genital Tract. sixth ed: Springer; 2011. p. 254-303.
21. Sedlis A, Bundy BN, Rotman MZ, Lentz SS, Muderspach LI, Zaino RJ. A randomized trial of pelvic radation therapy versus no further therapy in selected patients with stage IB carcinoma of the cervix after radical hysterectomy and pelvic lymphadenectomy: A Gynecologic Oncology Group Study. Gynecol Oncol. 1999;73(2):177-83. Epub 1999/05/18.

Supplemental Material 1: Diagnosis of myometrial invasion in more or less than 50% according to the initial diagnosis and the diagnosis after review of the histologic slides.



Supplemental Material 2: Adjusted hazard ratios (HR) of progression free survival by Depth Of myometrial Invasion <4mm or ≥4mm, Myometrial Invasion <50% or ≥50%, and Tumor Free Distance ≤7mm or >7mm using multivariable Cox regression.

Variable		HR	95%CI	HR	95%CI
Depth Of myometrial Invasion					
< 4mm	136	1.00	reference	1.00	reference
≥ 4mm	177	**3.15**	**1.16-8.56**	**10.35**	**1.23-86.93**
Myometrial Invasion					
< 50%	189	1.00	reference	NS	
≥ 50%	124	**2.15**	**1.01-4.58**		
Tumor Free Distance					
≤ 7mm	NS[&]			NS	
> 7mm					

[&]Not Selected

CHAPTER 5

A NEW TYPE ENDOMETRIAL CARCINOMA: ENDOMETRIOID HISTOLOGY WITH ATROPHIC ENDOMETRIUM AND POOR PROGNOSIS

Y.P. Geels M.D.[1], J.M.A. Pijnenborg M.D. Ph.D.[2], S.H. van den Berg- van Erp M.D. Ph.D.[3], J. Bulten M.D. Ph.D.[4], D.W. Visscher M.D. Ph.D.[5], S.C. Dowdy M.D. Ph.D.[6], L.F.A.G. Massuger M.D. Ph.D.[1]

[1]Department of Obstetrics and Gynecology, Radboud University Nijmegen Medical Centre, The Netherlands
[2]Department of Obstetrics and Gynecology, TweeSteden Hospital, Tilburg, The Netherlands
[3]Department of Pathology, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands
[4]Department of Pathology, Radboud University Nijmegen Medical Centre, The Netherlands
[5]Department of Anatomic Pathology, Mayo Clinic, Rochester, MN 55905, USA
[6]Division of Gynecologic Surgery, Mayo Clinic, Rochester, MN 55905, USA

Obstet Gynecol. 2012 Nov;120(5):1124-31

Abstract

Objective: Type I endometrial carcinomas are characterized by endometrioid histology, develop from hyperplastic endometrium, and have a good prognosis. Type II, non-endometrioid carcinomas, arise in atrophic endometrium, and have a poor prognosis. However, about 20% of cases do not fit within this dualistic model and include endometrioid carcinomas associated with recurrence, and possibly with atrophy. We aimed to evaluate grade 1 endometrioid endometrial carcinomas with atrophic endometrium, a putative third type endometrial carcinoma.

Methods: Histologic slides of all grade 1 endometrioid endometrial cancers from the Radboud University Medical Centre and Canisius-Wilhelmina Hospital from 1999-2009, and from the Mayo Clinic from 2002-2008 were reviewed. Comparisons were made between patients with atrophic and hyperplastic endometrium.

Results: After review, 527 patients were identified. In 88 patients (16.8%) background endometrium was atrophic, 387 patients (73.3%) had hyperplastic endometrium. Fifty-two patients (9.9%) had proliferative endometrium or no background endometrium and were excluded. Atrophy correlated with older age, low BMI, advanced FIGO-stage, malignant cells in peritoneal cytology, lymph node metastases, cervical involvement, lymphovascular space invasion, and deep myometrial invasion. Multivariable analysis showed that age (HR 1.06, 95%CI 1.01-1.12), FIGO stage (HR 8.47, 95%CI 1.73-41.57), and background endometrium(HR 3.11, 95%CI 1.11-8.70) were predictors of progression-free survival.

Conclusion: Atrophic endometrium is an independent prognostic factor for patients with grade 1 endometrioid endometrial carcinoma. Endometrioid carcinoma with atrophy may not follow the hypothesized progression model for type I tumors and may arise through unique carcinogenic pathways. Future research should investigate the carcinogenic pathways in this interesting group of patients.

Introduction

Cancer of the uterine corpus is the most common gynecologic malignancy in industrialized nations with an incidence of 43,470 and an estimated death rate of 7,950 annually in the United States.[1,2] A dualistic model for carcinogenesis in endometrial cancer has been accepted worldwide.[3-5] The majority of endometrial carcinomas are classified as type I carcinomas, and are related to unopposed estrogenic stimulation due to obesity, or exogenous hormone use. Type I endometrioid endometrial carcinomas occur in women at a median of sixty years of age, originate from hyperplastic endometrium, and generally have a good prognosis. In contrast, type II carcinomas include serous and clear cell histology, are unrelated to estrogenic stimulation, and occur in relatively older women. The majority of type II endometrial carcinomas arise in a background of atrophic endometrium, and generally have a poor prognosis.[6,7]

Distinct carcinogenic pathways underlie the observed clinical differences between type I and II endometrial cancer. Type I carcinomas are characterized by diploid tumors, expression of estrogen and progesterone receptors, *PTEN* alterations, microsatellite instability (MSI), and mutations of *KRAS* and *CTNNB1.* Type II carcinomas on the contrary, are often aneuploid, and show over expression of p53 and Her2/neu.[5-8,10]

Approximately 20% of cases do not fit within the dualistic model described above, and include patients with endometrioid endometrial carcinoma associated with recurrence and poor clinical outcome.[9,11] It has been suggested that a third endometrial cancer entity exists: endometrioid carcinomas arising in atrophic endometrium. This purported “type III endometrial cancer” is hypothesized to be associated with clinical outcomes intermediate between type I and type II lesions.[12] Furthermore, carcinogenesis in this group may occur through distinct mechanisms.

The aim of the current study was to estimate the clinical relevance of this hypothesized third type endometrial carcinoma. Therefore, we compared clinical and pathologic features in patients with hyperplastic and atrophic background endometrium in a large series of grade 1 endometrioid endometrial carcinoma patients. Second, we analyzed the prognostic impact of the background endometrium.

Materials and Methods

Patient selection

The Dutch nationwide network and registry of histology and cytopathology (Pathologisch Anatomisch Landelijk Geautomatiseerd Archief: PALGA) was used to search for all patients diagnosed, and surgically treated with at least hysterectomy and bilateral oophorectomy at the Radboud University Nijmegen, Medical Centre (RUNMC), and the Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands for primary grade 1 endometrioid endometrial carcinoma between January 1999 and January 2010. Subsequently, all patients diagnosed with primary grade 1 endometrioid endometrial carcinoma, and surgically treated with at least hysterectomy and bilateral oophorectomy in the Mayo Clinic, Rochester, Minnesota, USA, from January 2002 till January 2009 were included in the study. A total of 572 grade 1 endometrioid endometrial carcinoma patients were identified. This number included 143 patients from Nijmegen, and 429 patients from the Mayo Clinic. Clinical data were abstracted from patient records. Age, menopausal state, body mass index (BMI), parity, personal medical history, treatment, stage of disease, date of recurrence of disease, date of death, and the cause of death were registered. Stage of disease was based on the 2009 International Federation of Gynecology and Obstetrics (FIGO) staging system.[13] Patients with a personal history of any malignancy, or any synchronous primary malignancy were excluded from further analyses. Follow up data were extracted from the medical charts. In case of incomplete follow-up data, the patient or the physician was contacted. The median time of follow up was 50 months (range 0-128).

Review of the histologic slides

The slides of the primary carcinoma and the surrounding background endometrium of all patients were retrieved from the pathology archives and reviewed. Review was done systematically including the following items: the histologic type, tumor grade, depth of myometrial invasion (MI), the presence of lymphovascular space invasion (LVSI), and the nature of the background endometrium.[14] Review was performed in every hospital separately by an independent experienced pathologist (JB, SB, DV), who was unaware of the results of the original pathology reports or the clinical outcome of the patients. There was no systematic review between the centers. In case of doubt about the diagnosis, or in

case of discrepancy with the original pathology report a second review was performed by another experienced pathologist from the concerning hospital, and consensus about the diagnosis was made.

Background endometrium was grouped in eight categories: simple hyperplasia (SH), simple atypical hyperplasia (SAH), complex hyperplasia (CH), complex atypical hyperplasia (CAH), disordered proliferative endometrium, atrophic endometrium, normal proliferative endometrium, and determination of the background endometrium not possible.

Hyperplasia was defined as a proliferation of glands with an increase in gland:stroma ratio of 3:1 and a variety of abnormal architectural patterns.[15] Cytological atypia was defined as enlarged, rounded, polymorphic nuclei with loss of polarity, prominent nucleoli, chromatin clumbing, and an increased nuclear to cytoplasmic ratio.[6] Hyperplasia was categorized according to the World Health Organization (WHO) classification system for hyperplasia, which is based on the study of Kurman and colleagues.[16]

The endometrium was considered disordered proliferative when some of the present glands in postmenopausal women showed proliferative activity, and the gland:stroma ratio was slightly increased, but did not meet the hyperplasia criterion of 3:1.[6,15]

Atrophic endometrium was defined as shallow endometrium with a thin basalis, and with a few tubular glands lined by inactive epithelium.[17] In case of focal hyperplasia or focal disordered proliferative endometrium, the background endometrium was diagnosed as atrophic when more than 50% of the background endometrium was atrophic. In these cases the sub classification “mixed atrophy” was used, whereas cases without any proliferative glands were classified as “pure atrophy”.

In premenopausal women, proliferative endometrium was defined as widely spread, sometimes tortuous, tubular glands that showed mitotic activity, and abundant stroma.[17]

In some cases the tumor covered the entire endometrial cavity, so the nature of the background endometrium could not be determined.

The median number of slides available per patient in the cases with hyperplasia was four (range 1-23). For the patients with atrophic endometrium the median number of available slides was four as well (range 2-10).

Statistical analysis

Comparison was made between the group with atrophic and the group with hyperplastic background endometrium. The atrophy group consisted of patients with mixed and pure atrophy. The hyperplasia group consisted of patients with SH, SAH, CH, CAH, and disordered proliferative endometrium. When normal proliferative endometrium was found in premenopausal patients, or when no background endometrium could be found in the endometrial cavity, patients were excluded from analyses. Sub analyses were performed comparing pure atrophic background endometrium with mixed atrophic background endometrium. Differences in clinical and pathologic parameters between the group of patients with atrophic and hyperplastic endometrium were tested for statistical significance using the Pearson's chi-Square (χ^2) test, or the Fisher's exact test, and the Mann-Whitney test. The *P*-values presented are two-sided, and associations were considered significant if the *P*-value was less than 0.05. Survival techniques were used to study the progression free survival (PFS). PFS was calculated from the date of surgery until the date of recurrence. In case of no recurrence, the date of last contact or death was used for censoring. The prognostic impact of patient and tumor characteristics age, BMI, FIGO stage, peritoneal cytology, lymph node involvement, cervical involvement, lymphovascular space invasion, myometrial invasion, diameter of the tumor, and background endometrium were analyzed by using univariable and multivariable Cox proportional hazards models. The forward stepwise method was used for selection procedures for multivariable Cox proportional hazards models. These results were expressed as hazard ratio's (HR) with their 95% confidence intervals (95% CI). Statistical analyses were performed using the software package SPSS 18.0 for Microsoft Windows (SPSS Inc).

Ethical committee approval

For the cohort from the Mayo Clinic the study protocol was approved by the Institutional Review Board of Mayo Foundation; in accordance with the Minnesota Statute for Use of Medical Information in Research, only those patients who consented to the use of their medical records were included. For the cohort from the RUNMC and the Canisius-Wilhelmina Hospital, the Research Ethics Committee of the Radboud University Nijmegen

Medical Centre declared that the study protocol is in accordance with the applicable rules concerning the review of research ethics committees and informed consent.

Results

Patient characteristics

In total 572 grade 1 endometrioid endometrial carcinoma patients were identified. Thirty-two patients were excluded because of personal history of other malignancy. After review, thirteen cases were diagnosed with tumor grade 2 and were excluded. A total of 527 patients with grade 1 endometrioid endometrial carcinoma remained for analyses. Demographic and histopathologic characteristics of the 527 patients are shown in Table 1. As expected, the majority of patients were stage I and demonstrated favorable histologic characteristics. Fifty-nine patients received adjuvant therapy. In 21 patients recurrence of disease occurred, and only 7 patients died as a consequence of the disease. This results in a five year progression free survival rate of 96% and a five year overall survival rate of 99%. Note that lymph node dissection was omitted in a large proportion of the cohort. For the 429 patients from Mayo clinic, omission of lymph node dissection in 185 patients occurred as per protocol for patients with less than 50% myometrial invasion and tumor diameter less than 2cm as previously described.[18] For the 143 patients from Nijmegen, lymph node dissection was omitted in 137 cases without clinical suspicion of FIGO stage II or more, as recommended by the Dutch guidelines for endometrioid endometrial cancer treatment.[19]

Nature of the background endometrium

The type of background endometrium diagnosed in the total cohort is shown in Table 2. Some derivation of hyperplasia was present in 387 patients (73%) while atrophic endometrium was diagnosed in 88 patients (17%). In 25 patients (5%) there was extensive growth of the tumor in the endometrial cavity causing the entire endometrium to be substituted by the carcinoma. In these patients no background endometrium could be identified and were therefore excluded from analyses. Furthermore, in 27 patients (5%) normal premenopausal, proliferative endometrium was found. Since the hormonal regulated status of the premenopausal endometrium is not comparable to the non-stimulated status of the postmenopausal endometrium, these patients were also excluded from further analyses. An

Table 1: Clinical and pathologic characteristics of all grade 1 endometrioid endometrial carcinoma patients (N=527).

Variables		median / N	range/ %
Age at diagnosis (years) (N=527)		62	25-92
BMI (kg/m^2) (N=507)		32	16-76
FIGO Stage (2009)			
I	A	459	87.0
	B	54	10.2
II		3	0.6
III		10	2.0
IV		1	0.2
Hypertension			
No		243	46.1
Yes		282	53.5
Unknown		2	0.4
Diabetes Mellitus			
No		426	80.8
Yes		99	18.8
Unknown		2	0.4
Peritoneal Cytology			
Negative		370	70.2
Positive		32	6.1
Not sampled		125	23.7
Para-aortic and/or Pelvic Lymph nodes			
Negative		221	41.9
Positive	Pelvic	4	0.8
	Pelvic and para-aortal	1	0.2
Not sampled		301	57.1
Cervical involvement			
No		517	98.1
Yes	Endocervical glands	7	1.3
	Cervical stroma	3	0.6
Lymphovascular space invasion			
No		510	96.8
Yes		17	3.2

Myometrial invasion		
No invasion of myometrium	141	26.7
≤1/2 myometrium	324	61.5
>1/2 myometrium	62	11.8
Diameter of tumor (mm) (N=409)	25	1-145
Adjuvant therapy		
No	467	88.6
Radiotherapy	48	9.1
Chemotherapy	5	1.0
Chemotherapy + radiotherapy	6	1.1
Unknown	1	0.2
Recurrence of disease		
No	502	95.3
Yes	21	4.0
Unknown	4	0.7
Patient died		
No	494	93.7
Yes, as a consequence of disease	7	1.4
Yes, different cause	20	3.8
Yes, unknown cause	6	1.1

Table 2: Background endometrium in all endometrioid endometrial carcinoma patients (N=527).

	N	Per cent
Simple hyperplasia	36	6.8
Simple hyperplasia with atypia	10	1.9
Complex hyperplasia	7	1.3
Complex hyperplasia with atypia	308	5.4
Disordered proliferative endometrium	26	4.9
Total hyperplasia	**387**	**73.3**
Pure atrophy	44	8.4
Mixed atrophy	44	8.4
Total atrophy	**88**	**16.8**
Normal proliferative endometrium	27	5.1
No background endometrium	25	4.8
Total excluded from analyses	**52**	**9.9**
Total	**527**	**100**

example of endometrioid endometrial carcinoma with atrophic background endometrium, and endometrioid endometrial carcinoma with hyperplastic background endometrium is shown in Figure 1.

Results of analyses of atrophic background endometrium (N=88) and hyperplastic background endometrium (N=387) with clinical and pathologic characteristics are shown in Table 3. There were significant associations between atrophic endometrium and older age (P<0.01), and lower BMI (P<0.01). Furthermore, patients with atrophic endometrium were more likely to have advanced stage disease (P<0.01), malignant cells in peritoneal cytology (P=0.01), lymph node metastases (P=0.01), cervical involvement (P=0.03), lymphovascular space invasion (P<0.01), and deep myometrial invasion (P<0.01). Note that atrophic endometrium was present in all patients with metastatic lymph nodes. No differences were found between the atrophic and hyperplastic group with respect to hypertension, diabetes mellitus, and tumor diameter. When analyses were limited to patients with pure atrophic endometrium versus patients with mixed atrophic endometrium, a significant association was found only with pure atrophic endometrium and deep myometrial invasion (data shown in Supplemental Digital Content 1).

Figure 1: Atrophic and hyperplastic background endometrium.

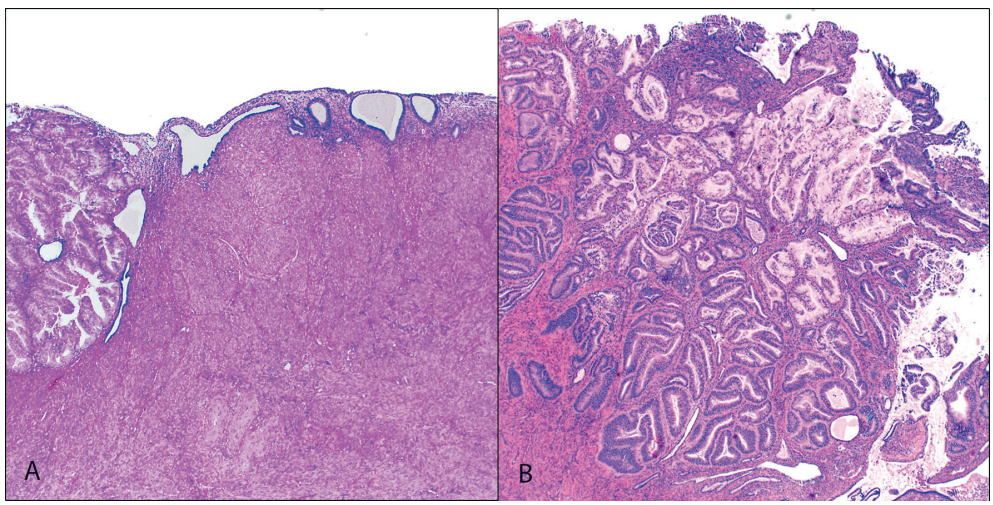


Figure 1A: Endometrioid endometrial carcinoma with atrophic endometrium
Figure 1B: Endometrioid endometrial carcinoma with hyperplastic endometrium

Table 3: Clinical and pathologic characteristics in patients with atrophic background endometrium compared with patients with hyperplastic background endometrium.

	Atrophy (N=88)		Hyperplasia (N=387)		
	mean/N	range/%	mean/N	range/%	*P*-value
Median age (years)	69	40-89	62	25-89	**<0.01**
Median BMI (kg/m^2)	30.9	20.0-66.0	33.5	16.0-76.0	**<0.01**
FIGO (2009)					
Stage I	82	93.2	383	99.0	**<0.01**
Stage II,III, IV	6	6.8	4	1.0	
Hypertension					
No	37	42.5	176	45.6	0.60
Yes	50	57.5	210	54.4	
Diabetes Mellitus					
No	74	85.1	308	79.8	0.26
Yes	13	14.9	78	20.2	
Peritoneal Cytology					
Negative	59	84.3	272	91.9	**0.01**
Positive	11	15.7	18	8.1	
Para-aortic and/or Pelvic lymph nodes					
Negative	41	93.2	154	100	**0.01**
Positive	3	6.8	0	0.0	
Cervical involvement (glands and/or stroma)					
No	84	95.5	384	99.2	**0.03**
Yes	4	4.5	3	0.8	
Lymphovascular space invasion					
Negative	81	92.0	380	98.2	**<0.01**
Positive	7	8.0	7	1.8	
Myometrial invasion					
<50%	68	77.3	359	92.5	**<0.01**
>50%	20	22.7	28	7.5	
Median diameter of tumor (mm)	28.0	1.0-90.0	24.0	1.0-110.0	0.06

Survival analysis

In a Cox proportional hazard model clinicopathologic variables including pre-existing endometrium were analyzed for their association with progression free survival. In univariable analyses age, BMI, FIGO stage, malignant cells in peritoneal cytology, lymph node metastases, lymphovascular space invasion, myometrial invasion, and atrophic endometrium were significantly associated with PFS (Table 4). No associations were found between PFS and cervical involvement, and diameter of the tumor. In multivariable analyses

Table 4: Crude hazard ratios (HR) of progression free survival (PFS) by clinicopathologic variables using univariable Cox regression.

Variable	N	HR	95%CI
Age at diagnosis in years	508	**1.07**	**(1.03-1.11)**
BMI* in kg/m^2	489	**0.94**	**(0.88-0.99)**
FIGO (2009)			
Stage I	508	1.00	reference
Stage II,III, IV	14	**10.98**	**(3.19-37.77)**
Peritoneal Cytology			
Negative	369	1.00	reference
Positive	31	**5.14**	**(1.95-13.55)**
Para-aortic and/or Pelvic lymph nodes			
Negative	220	1.00	reference
Positive	5	**21.38**	**(4.51-101.43)**
Cervical involvement (glands and/or stroma)			
No	513	1.00	reference
Yes	10	3.00	(0.40-22.54)
Lymphovascular space invasion			
Negative	507	1.00	reference
Positive	16	**5.39**	**(1.24-23.46)**
Myometrial invasion			
<50%	459	1.00	reference
>50%	63	**5.13**	**(2.12-12.41)**
Diameter of tumor (mm)	394	1.01	0.98-1.03
Background endometrium			
Atrophic	86	**5.37**	**(2.07-13.91)**
Hyperplastic	385	1.00	reference

only older age, advanced FIGO stage, and the presence of atrophic endometrium were independent predictors of PFS (Table 5). Furthermore, when comparing pure atrophic endometrium with mixed atrophic endometrium no significant difference in PFS was found (data shown in Supplemental Digital Content 2).

Table 5: Adjusted hazard ratios (HR) of progression free survival (PFS) by clinicopathologic variables using multivariable Cox regression.

Variable	N	HR	95%CI
Age at diagnosis in years	471	**1.06**	**(1.01-1.12)**
FIGO (2009)			
Stage I	461	1.00	Reference
Stage II,III, IV	10	**8.47**	**(1.73-41.57)**
Lymphovascular space invasion			
Negative		NS	
Positive			
Myometrial invasion			
<50%		NS	
>50%			
Background endometrium			
Atrophic	86	**3.11**	**(1.11-8.70)**
Hyperplastic	385	1.00	Reference

Discussion

In this study, the presence of atrophic endometrium in patients with grade 1 endometrioid endometrial carcinoma was associated with predictors of poor clinical outcome including high FIGO stage, lymphovascular space invasion, and deep myometrial invasion. Furthermore, atrophic endometrium was significantly associated with poor progression free survival in multivariable analyses.

The association with deep myometrial invasion and lymphovascular space invasion indicates more aggressive behavior of the tumors with atrophic background endometrium. The expression of this aggressive behavior can be found in the correlation with high FIGO stage, malignant cells in peritoneal cytology, and a poor progression free survival.

Atrophy has been associated with poor survival previously.[12] However, in the study by Sivridis and colleagues endometrioid endometrial carcinomas with tumor grades 1, 2, and 3 were included. The tumor grade is one of the most important predictors of recurrent disease in endometrioid endometrial carcinoma patients.[20,21] Grade 3 endometrioid endometrial carcinomas are a distinct, biologically more aggressive subtype, showing p53 expression in 17-57% of the cases.[22,23] It has been suggested that grade 3 endometrioid endometrial carcinoma is a type II endometrial cancer; furthermore, the diagnosis of serous carcinoma versus grade 3 endometrioid endometrial carcinoma is subject to a great deal of variability among pathologists. In addition, grade 3 endometrioid endometrial carcinomas are more often seen in a background of atrophic endometrium, whereas grade 1 endometrioid endometrial carcinomas more often have a background of hyperplastic endometrium.[7,24] Therefore, the difference in survival found by Sivridis and colleagues could be partly confounded by the inclusion of grade 3 tumors. In the current study, atrophic endometrium remained a significant predictor of poor PFS with multivariable analyses in a group limited to grade 1 endometrioid endometrial carcinoma patients.

The dualistic model for oncogenesis in endometrial cancer patients is not applicable for about 20% of individual cases who present with advanced disease, or recur despite the absence of risk factors. These cases with less favorable clinical outcomes may be represented by the cohort we describe here, namely patients with grade 1 endometrioid endometrial carcinoma and an atrophic background endometrium. In our study 17% of the patients had atrophic endometrium, a similar proportion of patients who do not fit into the

dualistic model.[9,11,12] These grade 1 endometrioid endometrial carcinomas may not follow the progression model for type I tumors with unopposed estrogenic stimulation resulting in hyperplasia followed by transition to endometrioid carcinoma.[6,15,25] The fact that we find grade 1 endometrioid endometrial carcinomas in atrophic endometrium, the correlation of atrophic endometrium with predictors of poor clinical outcome, and the correlation of atrophy with poor PFS indicate that carcinogenesis in these cases may occur through distinct mechanisms.

Many molecular markers have been identified to emphasize the difference between type I and type II endometrial cancer. Type I tumors are characterized by estrogen- and progesterone receptor expression, Micro Satellite Instability (MSI), *PTEN* alterations, and mutations of *KRAS* and *CTNNB1,* whereas the majority of type II carcinomas have p53 mutations and her-2/neu amplifications.[8,10] The considerable number of molecular changes identified in type I endometrial carcinomas make it less likely that one pathway will fit all individual cases. Future endometrial cancer research should compare the immunohistochemical and molecular appearance of endometrial cancer patients with atrophic and hyperplastic background endometrium.

The majority of postmenopausal women have atrophic endometrium.[26] However, while hyperplastic endometrium is generally a result of diffuse estrogenic stimulation of the entire endometrium, focal proliferation and hyperplasia have been described.[15] Furthermore, weakly proliferative endometrium has been reported in disease free postmenopausal women in half of the cases in one study.[27] These results are comparable with the results found in our study; 50% of the carcinoma patients with atrophic endometrium show proliferation to some extent in the endometrium. The arbitrarily chosen cut off point of more than 50% atrophic endometrium was used to categorize the background endometrium showing these ambiguous features of atrophy with focal proliferation. When comparing patients with mixed atrophic background endometrium versus patients with pure atrophic background endometrium, few differences in clinical and pathologic characteristics were found, and no difference in PFS was found, indicating that both groups are comparable.

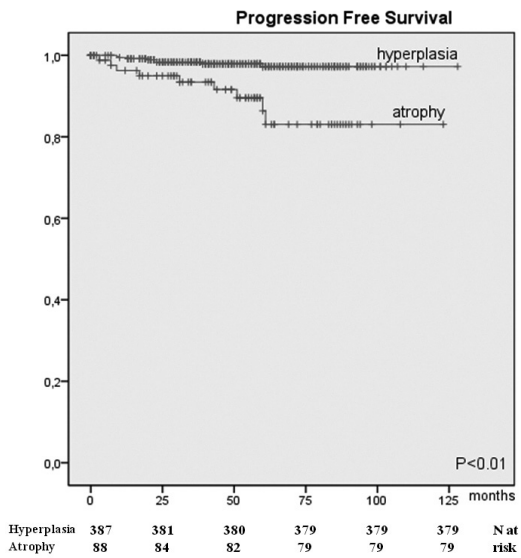
Proliferative endometrium is present in premenopausal women in the proliferative phase of the menstrual cycle. Proliferative endometrium is stimulated by estrogens, but it is not caused by unopposed estrogen excess.[17] This condition is comparable neither to the non-stimulated atrophic endometrium, nor to unopposed stimulated, hyperplastic

endometrium.[6] Therefore, patients with proliferative premenopausal endometrium were excluded from analyses.

This is a multicentre study including a large number of patients. Although it is retrospective with the inherent limitations of selection-bias and missing data, we were able to collect complete clinical data in the vast majority of the patients. All histologic slides were reviewed by three pathologists separately in the relating hospitals where the patients were treated. Criteria for the diagnosis of atrophy, hyperplasia, or disordered proliferative endometrium were set clearly before the start of the study, and were followed systematically by all pathologists.

In conclusion, we found atrophic background endometrium to be an independent prognostic factor in this large series of grade 1 endometrioid endometrial carcinoma patients. Endometrioid endometrial carcinoma with a background of atrophic endometrium may not follow the progression model for type I tumors, which normally arise in a background of hyperplasia. The nature of the background endometrium should be mentioned in every pathology report since it provides important prognostic information. Future research should investigate possible differences in carcinogenic pathways in these patients with atrophy and endometrioid endometrial carcinoma.

Figure 2: Kaplan-Meier survival analysis showing differences in progression-free survival in patients with atrophic and hyperplastic background endometrium.



References

1. Global Cancer Facts & Figures. 2008.
2. Cancer Facts & Figures. 2010.
3. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol 1983; 15(1): 10–7.
4. Sherman ME, Bur ME, Kurman RJ. p53 in endometrial cancer and its putative precursors: vidence for diverse pathways of tumorigenesis. Hum Pathol 1995; 26(11): 1268–74.
5. Sherman ME. Theories of endometrial carcinogenesis: a multidisciplinary approach. Mod Pathol 2000; 13(3): 295–308.
6. Ellenson HL, Ronnett BM, Kurman RJ. Precursor lesions of endometrial carcinoma. In: Kurman RJ, Ellenson HL, Ronnett BM, editors. Blaustein's Pathology of the Female Genital Tract. Sixth ed. New York: Springer; 2011. p. 359–91.
7. Ellenson HL, Ronnett BM, Soslow RA, Zaino RJ, Kurman RJ. Endometrial carcinoma. In: Kurman RJ, Ronnett BM, Ellenson HL, editors. Blaustein's Pathology of the Female Genital Tract. Sixth ed. New York: Springer; 2011. p. 393–452.
8. Markova I, Pilka R, Duskova M, Zapletalova J, Kudela M. Prognostic significance of clinic pathological and selected immunohistochemical factors in endometrial cancer. Ceska Gynekol 2010; 75(3): 193–9.
9. Engelsen IB, Akslen LA, Salvesen HB. Biologic markers in endometrial cancer treatment. APMIS. 2009; 117(10): 693–707.
10. Prat J, Gallardo A, Cuatrecasas M, Catasus L. Endometrial carcinoma: pathology and genetics. Pathology 2007; 39(1): 72–87.
11. Lax SF. Molecular genetic changes in epithelial, stromal and mixed neoplasms of the endometrium. Pathology 2007; 39(1): 46–54.
12. Sivridis E, Fox H, Buckley CH. Endometrial carcinoma: two or three entities? Int J Gynecol Cancer 1998; 8: 183–8.
13. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Int J Gynaecol Obstet 2009; 105(2): 103–4.
14. Silverberg SG, Mutter GL, Kurman RJ, Kubik-Huch RA, Nogales F, Tavassoli FA. Tumors of the uterine corpus: epithelial tumors and related lesions. In: avassoli FA SF, editors, editor. WHO classification of tumors: pathology and genetics of tumors of the breast and female genital organs. 5th ed: World Health Organisation; 2003. p. 221–32.
15. Mills AM, Lonacre TA. Endometrial hyperplasia. Seminars in Diagnostic Pathology 2010; 27: 199–214.
16. Kurman RJ, Kaminski PF, Norris HJ. The behavior of endometrial hyperplasia. A long-term study of "untreated" hyperplasia in 170 patients. Cancer 1985; 15; 56(2): 403–12.
17. McCluggage WG. Benign Diseases of the Endometrium. In: Kurman RJ, Ellenson LH, Ronnett BM, editors. Blaustein's Pathology of the Female Genital Tract. 6th ed: Springer; 2011. p. 305-58.

18. Mariani A, Dowdy SC, Cliby WA, Gostout BS, Jones MB, Wilson TO, et al. Prospective assessment of lymphatic dissemination in endometrial cancer: a paradigm shift in surgical staging. Gynecol Oncol 2008 Apr; 109(1) :11–8.
19. www.oncoline.nl. 2011.
20. Creutzberg CL, van Putten WL, Warlam-Rodenhuis CC, van den Bergh AC, de Winter KA, Koper PC, et al. Outcome of high-risk stage IC, grade 3, compared with stage I endometrial carcinoma patients: the Postoperative Radiation Therapy in Endometrial Carcinoma Trial. J Clin Oncol 2004: 1; 22(7): 1234–41.
21. Fujimoto T, Nanjyo H, Fukuda J, Nakamura A, Mizunuma H, Yaegashi N, et al. Endometrioid uterine cancer: histopathological risk factors of local and distant recurrence. Gynecol Oncol 2009; 112(2):342–7.
22. Halperin R, Zehavi S, Habler L, Hadas E, Bukovsky I, Schneider D. Comparative immunohistochemical study of endometrioid and serous papillary carcinoma of endometrium. Eur J Gynaecol Oncol 2001; 22(2): 122–6.
23. Lax SF, Kendall B, Tashiro H, Slebos RJ, Hedrick L. The frequency of p53, K-ras mutations, and microsatellite instability differs in uterine endometrioid and serous carcinoma: evidence of distinct molecular genetic pathways. Cancer 2000; 15; 88(4): 814–24.
24. Voss MA, Ganesan R, Ludeman L, McCarthy K, Gornall R, Schaller G, et al. Should grade 3 endometrioid endometrial carcinoma be considered a type 2 cancer-A clinical and pathological evaluation. Gynecol Oncol 2012; 124(1): 15–20.
25. Kurman RJ, McConnell TG. Precursors of endometrial and ovarian carcinoma. Virchows Arch 2010; 456(1): 1–12.
26. Gol K, Saracoglu F, Ekici A, Sahin I. Endometrial patterns and endocrinologic characteristics of asymptomatic menopausal women. Gynecological endocrinology 2001; 15(1): 63–7.
27. Sivridis E, Giatromanolaki A. Proliferative activity in postmenopausal endometrium: the lurking potential for giving rise to an endometrial adenocarcinoma. J Clin Pathol 2004; 57(8): 840–4.

Supplemental Digital Content 1: Clinical and pathologic characteristics in patients with pure atrophic background endometrium compared with patients with mixed atrophic background endometrium.

	Pure atrophy (N=44)		Mixed atrophy (N=44)		*P*-value
	N	(%)	N	(%)	
Median age in years (range)	69 (40-85)		70 (48-89)		0.37
Median BMI in kg/m^2 (range)	30.9 (20.0-55.0)		31.0 (20.0-66.0)		0.87
Hypertension					
No	20	46.5	17	38.6	0.46
Yes	23	53.5	27	61.4	
Diabetes Mellitus					
No	34	79.1	40	90.9	0.14
Yes	9	20.9	4	9.1	
FIGO (2009)					
Stage I	41	93.2	41	93.2	1.00
Stage II,III, IV	3	6.8	3	6.8	
Peritoneal Cytology					
Negative	32	88.9	27	45.8	0.34
Positive	4	11.1	7	63.6	
Para-aortic and/or Pelvic lymph nodes					
Negative	19	95.0	22	53.7	1.00
Positive	1	5.0	2	66.7	
Cervical involvement (glands and/or stroma)					
No	40	90.9	44	52.4	0.17
Yes	4	9.1	0	0.0	
LVSI					
Negative	43	93.2	40	90.9	1.00
Positive	1	6.8	4	9.1	
Myometrial invasion					
<50%	30	68.2	38	86.4	**0.04**
>50%	14	31.8	6	13.6	
Diameter of tumor (mm)	34.5 (11.0-85.0)		25.0 (1.0-90.0)		0.15

Supplemental Digital Content 2: Crude hazard ratios (HR) of progression free survival (PFS) by mixed atrophy vs. pure atrophy using univariable Cox regression.

Variable	N	HR	95%CI
Background endometrium			
Mixed atrophy	43	1.00	reference
Pure atrophy	43	0.88	0.24-3.30

CHAPTER 6

IMMUNOHISTOCHEMICAL AND GENETIC PROFILE OF ENDOMETRIOID ENDOMETRIAL CARCINOMA ARISING FROM ATROPHIC ENDOMETRIUM

Yvette P. Geels, MD[1], Louis J.M. van der Putten, MD[1], Angela A.G. van Tilborg, PhD[1,2], Irene Lurkin, BSc[3], Ellen C. Zwarthoff, PhD[3], Johanna M.A. Pijnenborg, MD, PhD[4], Saskia H. van den Berg-van Erp, MD, PhD[5], Marc P.L.M. Snijders, MD, PhD[6], Johan Bulten, MD, PhD[2], Daniel W. Visscher, MD, PhD[7], Sean C. Dowdy, MD[8], Leon F.A.G. Massuger, MD, PhD[1]

[1]Department of Obstetrics and Gynecology, Radboud university medical center, Nijmegen, The Netherlands
[2]Department of Pathology, Radboud university medical center, Nijmegen, The Netherlands
[3]Department of Pathology, Erasmus MC, Rotterdam, The Netherlands
[4]Department of Obstetrics and Gynecology, TweeSteden Hospital, Tilburg, The Netherlands
[5]Department of Pathology, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands
[6]Departement of Obstetrics and Gynecology, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands
[7]Department of Anatomic Pathology, Mayo Clinic, Rochester, Minnesota, United States
[8]Division of Gynecologic Surgery, Mayo Clinic, Rochester, Minnesota, United States

Submitted

Abstract

Objective: Endometrial carcinoma is divided into type I endometrioid endometrial carcinoma (EEC), the majority of which arises from hyperplastic endometrium, and type II nonendometrioid endometrial carcinoma (mainly serous histology), the majority of which arises from atrophic endometrium. However, a minority (20%) of EECs are found to have an atrophic background endometrium, which was found to be a marker of a worse prognosis. The aim of the present study was to compare the immunohistochemical and genetic profile of this possible third type to that of the known two types.

Methods: 43 patients with grade 1 EEC and hyperplastic background endometrium (type I), 43 patients with grade 1 EEC and atrophic background endometrium (type III) and 21 patients with serous carcinoma (type II) were included (N=107). Tissue microarrays of tumor samples were immunohistochemically stained for PTEN, L1CAM, ER, PR, p53, MLH1, PMS2, β-catenin, E-cadherin, and MIB1. The *BRAF, KRAS,* and *PIK3CA* genes were analyzed for mutations.

Results: A significantly higher expression of ER and PR, and lower expression of L1CAM, p53, and MLH1 in type I and III compared to type II carcinomas was seen. Expression of E-cadherin was significantly lower in type III compared to type I carcinomas. Mutation analysis showed significantly less mutations of the *KRAS* gene in type III compared to type I and II carcinomas ($P<0.01$).

Conclusion: There appears to be only slight immunohistochemical and genetic differences between EEC with hyperplastic and with atrophic background endometrium. Carcinogenesis of EEC in atrophic endometrium is to be characterized by loss of E-cadherin and a lack of *KRAS* mutations. As expected, there were many significant differences in immunohistoschemical expression profiles between endometrioid and serous carcinomas.

Introduction

Cancer of the uterine corpus is the most common gynecologic malignancy among women in the developed world. In 2012, it affected 47,130 women and caused the death of 8,010 women in the US.[1]

It is generally accepted that endometrial carcinoma (EC) can be divided into two subtypes.[2] Type I endometrial carcinoma is the most common subtype. It affects women at a median age of 60 years, and has a good prognosis. These tumors usually are related to unopposed estrogen stimulation, and show endometrioid histology, arising from hyperplastic endometrium. In contrast, the less common type II carcinomas affect older women, and have a poor prognosis. These tumors are not related to unopposed estrogen stimulation, and are characterized by clear cell or serous histology, arising from atrophic endometrium.[3-5] Distinct carcinogenic pathways have been described in each subtype. Type I carcinomas are characterized by microsatellite instability and alterations of the *PTEN*, *KRAS, PIK3CA,* and *CTNNB1* genes, whereas type II carcinomas are often aneuploid and show over expression of p53 and Her2/neu.[6-9]

However, some tumors do not fit within this dualistic model. In a recent study we reviewed slides from 527 patients with grade 1 endometrioid endometrial carcinomas, and found that 17% of these carcinomas had atrophic background endometrium.[10] Furthermore, the presence of atrophic background endometrium adjacent to EEC was associated with several predictors of poor survival, and an independent predictor of reduced progression free survival in endometrioid endometrial carcinomas.

The aim of the present study was to analyze the hypothesis that endometrioid endometrial carcinoma with a background of atrophic endometrium arises through different carcinogenic pathways than type I and II endometrial carcinomas. Therefore, the expression patterns of several immunohistochemical markers and the presence of distinct genetic mutations in endometrioid endometrial carcinoma with a background of atrophic endometrium was compared to that of type I and II carcinomas.

Materials and methods

Patients

For this study, patients with endometrial carcinoma from two cohorts, at least treated with a hysterectomy and bilateral salpingo-oophorectomy and who did not have a personal history of malignancy, were evaluated for inclusion. The first cohort is comprised of patients treated for grade 1 endometrioid endometrial carcinoma at the Radboud University Nijmegen, Medical Center and the Canisius-Wilhelmina Hospital in Nijmegen, The Netherlands, between January 1999 and December 2009, and at the Mayo Clinic in Rochester, Minnesota, USA, between January 2002 and December 2008.[10] The second cohort is comprised of patients with uterine serous carcinoma treated at the Radboud University Nijmegen, Medical Center and the Canisius-Wilhelmina Hospital, Nijmegen between January 1999 and December 2009.[11,12]

Slides of the primary carcinoma and background endometrium from the cohort of patients with grade 1 endometrioid endometrial carcinoma were reviewed with special attention to the nature of the background endometrium by experienced pathologists (JB, SB, DV), who were unaware of the original pathology results and clinical outcome. In case of doubt or discrepancy with the original pathology report, a second review was performed by another pathologist and consensus was reached. Background endometrium was categorized as simple hyperplasia, simple atypical hyperplasia, complex hyperplasia, complex atypical hyperplasia, disordered proliferative, atrophic, and normal proliferative. These definitions are well described in literature and are summarized in Table 1.[5,10,13-15] Some cases had to be excluded because the tumor covered the entire cavity of the uterus and there was no background endometrium to be evaluated.

All patients with grade I endometrioid endometrial carcinoma and a background of pure (100%) atrophic endometrium (abbreviated as type III) as well as a similar amount of patients with grade I endometrioid endometrial carcinoma and a background of hyperplastic endometrium (type I) were included. Subsequently, all patients from the uterine serous carcinoma (type II) cohort of whom there was uterine tissue available were included. This cohort consisted of both pure and mixed serous histology. It has been described previously that about half of the serous carcinomas have pure serous histology.[16]

Table 1. Definitions used in characterizing the background endometrium.

Definition	Criteria
Hyperplasia	Gland proliferation, increased gland:stroma ratio of 3:1, a variety of abnormal architectural changes
Simple or complex	As defined by the WHO and Kurman *et al* [14]
Atypical	Enlarged, rounded, polymorphic nuclei with loss of polarity, prominent nucleoli, chromatin clumping, increased nuclear:cytoplasmatic ratio
Atrophic	Shallow endometrium with thin basalis, few tubular glands lined by inactive endometrium
Mixed	>50% atrophy
Pure	100% atrophy
Disordered proliferative	Some gland proliferation, but no hyperplasia. In postmenopausal women
Normal proliferative	Widely spread, sometimes tortuous, tubular glands showing mitotic activity and abundant stroma. In premenopausal women

Tissue microarray and immunohistochemistry

Tissue microarrays were created from the primary carcinoma.[17] Two representative areas of the carcinoma were selected on hematoxylin and eosin-stained slides. For the type II cases, areas with pure serous histology were selected. Two cylinders with a diameter of 2 mm were punched out of every donor block from the selected areas and mounted into a recipient paraffin block using a manual tissue microarrayer (Tissue-Tek, Quick-Ray, Sakura Finetek, USA).

The tissue microarrays were cut in 4 µm slides and immunohistochemically stained. Several markers were selected to be stained, based on the difference in their expression in type I and type II endometrial carcinoma [6-9, 18, 19]. An overview of the antibodies used in this study is shown in Table 2.

Immunohistochemical analysis of PTEN, L1CAM, ER, PR, p53, MLH1, PMS2, β-catenin, E-cadherin, and MIB1 expression was performed according to local protocols. These markers were chosen because previous literature has shown that their expression is different in type I and II EC [8, 9, 19]. In short, formalin fixed paraffin sections were stained with the primary antibody following EDTA antigen retrieval, blocking of endogenous background with Peroxidase Blocking Reagent and protein blocking using horse serum. Subsequently,

Table 2: Antibodies used in this study.

	Antibody	Company
PTEN inactivation	6H2.1	Dako*
L1CAM	UJ127	Thermo Scientific†
ER expression	SP1	Thermo Scientific
PR expression	PgR 636	Dako
P53 mutations	DO-7	Thermo Scientific
Loss of MLH1	G168-15	BD‡
Loss of PMS2	A16-4	BD
β-catenin alteration	14/Beta-Catenin	BD
E-cadherin alteration	SPM471	Thermo Scientific
High proliferation rate	MIB1	Thermo Scientific

*Dako , Glostrup, Denmark; † Thermo Fisher Scientific Inc., Waltham, Massachusetts, US; ‡ Becton, Dickinson and Company, Franklin Lakes, New Jersey, US

a secondary antibody was added and visualization was performed with Vectastain and 3,3'-Diaminobenzidine (Zymed lab. California, USA) as substrate. Staining was enhanced in $CuSO_4$ and slides were counterstained with Mayer's haematoxylin. Finally, slides were dehydrated and mounted.

Tumor samples were given a score ranging from 0-9 by two independent evaluators (YG, AT), which was the product of the percentage of cells stained (0%=0; 1-10%=1; 11-50%=2; 51-100%=3) and intensity of staining (none=0; weak=1; moderate=2; strong=3).[20] The evaluators were unaware whether the tissue cylinders were from type I, type II or type III carcinomas. Samples with too little tissue to assess or samples not containing any malignant tissue were not included in the calculations. In case of a large discrepancy between the score of the two evaluators (i.e. a difference in percentage or intensity score >2 or disagreement on the presence of malignant tissue) a third independent reviewer (JB), who was unaware of the score given by the first evaluators, scored the sample as well.

The final score per case (range 0-9) was calculated by adding all scores given to the two tissue samples and dividing them by the number of scores in the sum (which varied depending on the presence of tumor tissue in the sample and the need for a third review).

Mutation analysis

Slides with at least 10% representative tumor tissue were selected for DNA isolation. For the cases from the Mayo Clinic, the two TMA tumor biopsies were used instead. DNA was isolated with TET-lysis buffer (10 mmol/L Tris-HCl, pH 8.5; 1 mmol/L EDTA, pH 8; 0.1% Tween-20) containing 5% Chelex-100 (Bio-Rad, Hercules, CA). Protein digestion was performed by adding proteinase K to each sample and incubation at 56°C for 48 hours. Next, Protein K was inactivated at 95°C for ten minutes. The samples were centrifuged for ten minutes at 14.000 rpm (RT) and the DNA concentration of the supernatant was measured using the Quant-it picogreen dsDNA assay kit (Invitrogen, Carlsbad, California, U.S.) before storage at 4°C. For the detection of mutations, DNA was amplified for exons of the *KRAS, BRAF* and *PIK3CA* genes using earlier published PCR primers.[21] The amplified exons were assessed for mutations at 22 nucleotide positions by single nucleotide probe extension assays using a SNaPshot Multiplex kit (Applied Biosystems, Foster City, CA) as described previously.[21,22]

Statistical analysis

Differences between the three subtypes of endometrial carcinoma in marker scores were calculated using the Mann-Whitney-U test. The differences between the subtypes of endometrial carcinoma in the amount of cases with genetic mutations were calculated using the Chi square test and the Fisher's exact test. *P*-values <0.05 were considered significant. IBM SPSS 20.0 statistical software was used for analysis of the data.

Results

Patients

Of the 527 patients with grade 1 endometrioid endometrial carcinoma, background endometrium was hyperplastic in 387 (73%) and atrophic in 88 (17%). The background endometrium was normal premenopausal, proliferative in 27 (5%) patients and could not be assessed due to the size of the tumor in 25 (5%) patients. Pure atrophy was found in 43 (48.9%) of the 88 patients with atrophic background endometrium. Out of the 387 patients with hyperplastic background endometrium, 43 (11.1%) were randomly selected as controls. From the cohort of patients with uterine serous carcinoma, all patient data as well as tissue was present in 21 cases (out of 47 meeting the inclusion criteria), 14 (66.7%) of which had

pure serous histology, which is slightly higher than in the general population of patients with uterine serous carcinoma.[16] In total, 107 cases were included for immunohistochemical and mutation analysis. Patient and tumor characteristics per type are shown in Table 3.

Immunohistochemistry

After initial evaluation of 2140 tissue samples, additional review because of discrepancy between the evaluator's scores was necessary for 107 samples (44 type I, 19 type II and 44 type III). This was not significantly more or less for one of the markers. Three type III cases were excluded from calculations because both tumor samples contained too little representative tissue or because they did not contain malignant tissue (one ER case, one MLH1 case, and one MIB1 case). The final marker scores per type of endometrial carcinoma and the differences between the scores are shown in Figure 1.

When looking at the differences between the subtypes it can be seen that in both type I and III EC expression of ER and PR was significantly higher than in type II EC, while expression of L1CAM, p53 and MLH1 was significantly lower in type I and III EC than in type II EC. Expression of E-cadherin was significantly lower in type III EC compared to type I and II EC. An overview of the markers with significant differences in expression is shown in Figure 2.

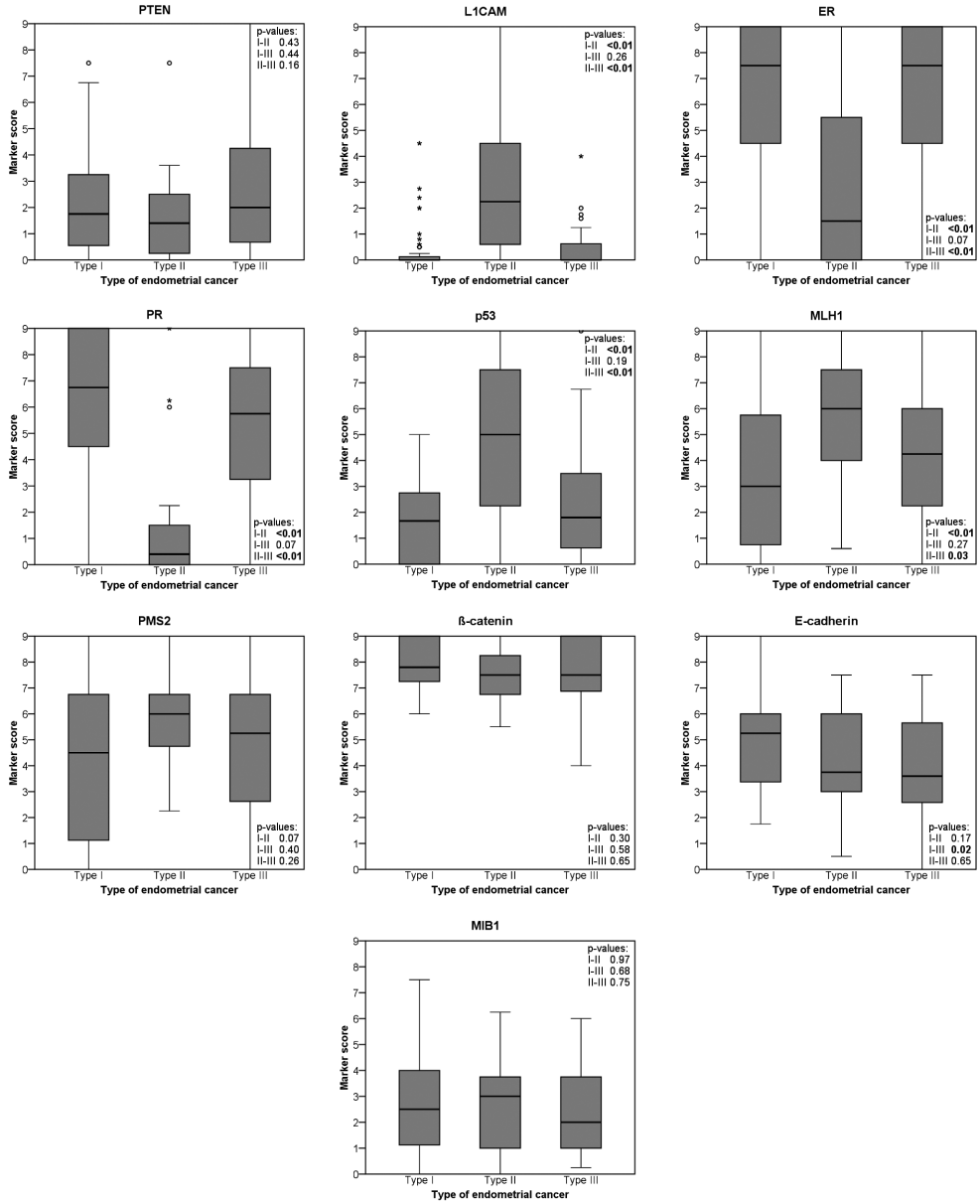
Mutation analysis

A representative part of the tumor could be retrieved for every case and DNA yield was adequate for every sample when assessed by spectral photometry. Mutation analysis of the *PIK3CA* gene was successful in all cases, of the *BRAF* gene in all but one type I case, and the *KRAS* gene in all but two type I, one type II, and two type III cases. The distribution of wild type and mutated genes per type as well as the differences between types is shown in Table 4. The only significant difference was found with the *KRAS* gene, which was mutated in 37.2% of the type I and 23.8% of the type II carcinomas, compared to only 2.3% of the type III carcinomas. The *BRAF* gene was not mutated in any of the cases, while the *PIK3CA* was mutated more often in type II EC (23.8%) than in type I (14%) and III (11.6%) EC, but these differences were not significant.

Table 3: Patient and tumor characteristics.

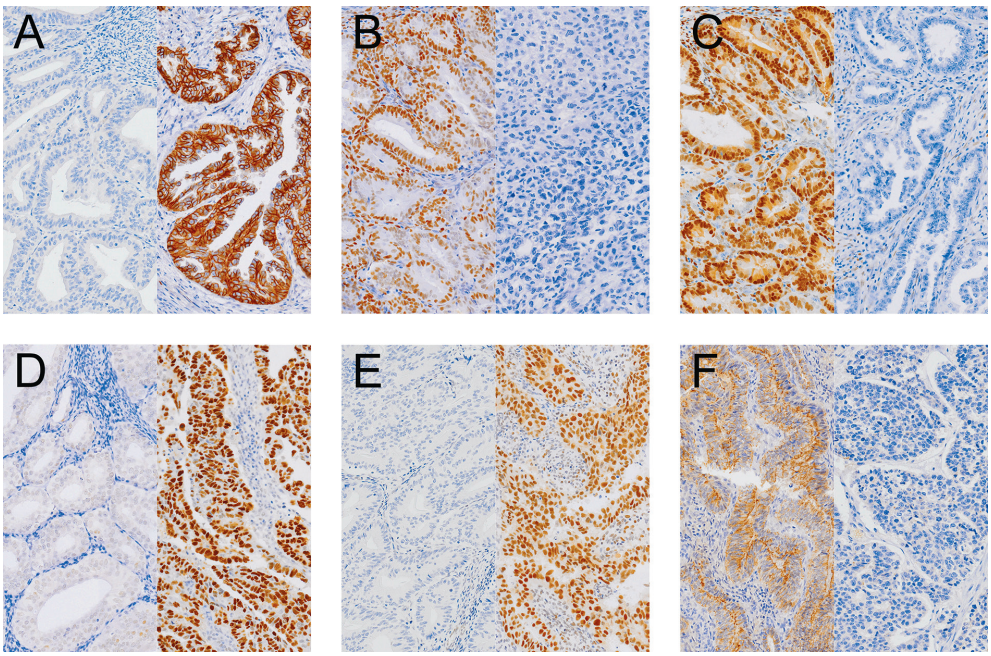
	Type I N (SD/%)	Type II N (SD/%)	Type III N (SD/%)
Age (years)	62 (SD10.3)	70 (SD9.6)	68 (SD10.1)
BMI (kg/m^2)	33 (SD8.1)	31 (SD7.4)	31 (SD7.4)
Hypertension			
Yes	20 (46.5)	7 (33.3)	23 (53.5)
No	22 (51.2)	13 (61.9)	19 (44.2)
Unknown	1 (2.3)	1 (4.8)	1 (2.3)
Diabetes			
Yes	10 (23.3)	3 (14.3)	9 (20.9)
No	32 (74.4)	17 (81)	33 (76.7)
Unknown	1 (2.3)	1 (4.8)	1 (2.3)
Surgical approach			
Minimally invasive	6 (14)	-	6 (14)
Laparotomy	16 (37.2)	21 (100)	16 (37.2)
Unknown	21 (48.8)	-	21 (48.8)
Lymphadenectomy			
Negative	10 (23.3)	10 (47.6)	19 (44.2)
Positive	-	5 (23.8)	1 (2.3)
Not sampled	33 (76.7)	6 (28.6)	23 (53.5)
Cervical involvement			
Endocervical	2 (4.7)	2 (9.5)	2 (4.7)
Stromal	-	9 (42.9)	2 (4.7)
No	41 (95.3)	10 (47.6)	39 (90.7)
LVSI*			
Yes	2 (4.7)	12 (57.1)	3 (7)
No	41 (95.3)	9 (42.9)	40 (93)
Myometrial invasion			
<50%	41 (95.3)	8 (38.1)	30 (69.8)
≥50%	2 (4.7)	13 (61.9)	13 (30.2)
FIGO stage			
IA	38 (88.4)	4 (19)	28 (65.1)
IB	4 (9.3)	2 (9.5)	9 (20.9)
II	-	3 (14.3)	2 (4.7)
III	1 (2.3)	5 (23.8)	4 (9.3)
IV	-	7 (33.3)	-

Figure 1: Boxplots showing the different marker scores per type of endometrial carcinoma.



P-values were calculated using the Mann-Whitney Test.

Figure 2: Overview of markers with a significant difference in expression between the subtypes of endometrial carcinoma.



Shown are examples of L1CAM (A), ER (B), PR (C), p53 (D) and MLH1 (E) expression in type I (left) and type II (right) endometrial carcinoma as well as examples of E-cadherin (F) expression in type I (left) and type III (right) endometrial carcinoma.

Table 4: Results of the mutation analysis per type of endometrial carcinoma.

	Type I N (%)	Type II N (%)	Type III N (%)	*P* (I-II)*	*P* (I-III)*	*P* (II-III)*
BRAF						
Wild type	42 (97.7)	21 (100)	43 (100)	-	-	-
Mutated	-	-	-			
Unknown	1 (2.3)	-	-			
KRAS						
Wild type	26 (60.5)	14 (66.7)	40 (93)	0.58	**<0.01**	**<0.01**
Mutated	15 (34.9)	6 (28.6)	1 (2.3)			
Unknown	2 (4.7)	1 (4.8)	2 (4.7)			
PIK3CA						
Wild type	37 (86)	16 (76.2)	38 (88.4)	0.48	1.00	0.28
Mutated	6 (14)	5 (23.8)	5 (11.6)			
Unknown	-	-	-			

**P*-value for the Fisher's exact test

Discussion

In this study we have analyzed the immunohistochemical and genetic profiles of endometrioid endometrial carcinoma with a background of hyperplastic (type I) or atrophic (type III) endometrium, which were quite comparable. However, endometrioid carcinomas with atrophic background endometrium showed less expression of E-cadherin and fewer mutations in *KRAS* compared to endometrioid carcinomas with hyperplastic background endometrium. As expected, when comparing carcinomas with endometrioid histology to those with serous histology (type II), they had a different immunohistochemical profile.

The immunohistochemical differences between type I and II endometrial carcinoma in this study are in line with previous literature.[8,9,19] As expected, ER and PR expression were significantly higher in type I carcinomas, while L1CAM, p53 and MLH1 were significantly higher in type II carcinomas. Moreover, while the differences in PMS2, E-cadherin, β-catenin and MIB1 expression were not significant, the observed trends were according to the findings in previous literature on these markers. Interestingly, only the results of PTEN

expression were not in line with previous literature, as it was surprisingly found to be lowest in serous carcinomas, while a loss of PTEN is expected in endometrioid carcinomas.

There were no *BRAF* gene mutations, regardless of the subtype, which is in line with the low *BRAF* mutation rate previously described in endometrial carcinoma.[23] *PIK3CA* mutations have been extensively shown to be present in both endometrioid and non-endometrioid endometrial carcinomas and have been associated with invasive growth and poor prognosis.[24-27] Indeed, in our study we found the *PIK3CA* mutation rate to be highest in type II carcinomas.

Mutations of the *KRAS* proto-oncogene have been described in up to 30% of endometrioid endometrial carcinomas, but in only up to 10% in type II carcinomas.[9] These mutations have been found to be an early event in the carcinogenesis of endometrioid endometrial carcinomas, present in an equal amount of endometrial hyperplasias and carcinomas.[28] We found a slightly higher amount of mutations in type I carcinomas than previously reported, but the discrepancy between previously published results and the amount of mutations we observed in type II carcinomas was much larger. As a representative sample of type II cases was included in this study, several of them had a minor component with non-serous histology.[16] Three of the six type II cases with mutations in the *KRAS* gene had a minor endometrioid component and the *KRAS* mutations might not have been present in the areas with serous histology, but in those with endometrioid histology.[28]

It was hypothesized that type III carcinomas would have a distinct immunohistochemical and genetic pattern when compared to type II carcinomas, and most interestingly, when compared to type I carcinomas. However, for most immunohistochemical markers, there was no difference in expression between type I and III carcinomas. Only the expression of E-cadherin was significantly lower in type III compared to type I carcinomas. Furthermore, the amount of mutations in the *KRAS* gene was significantly lower in type III compared to type I and II carcinomas.

In epithelial cells, E-cadherin is the major molecule of the cadherin family, which is essential for tight cell-cell connections.[29] Loss of E-cadherin by methylation of the E-cadherin gene has been described in grade 3 endometrioid as well as in non-endometrioid carcinomas, but not in hyperplastic endometrium. Loss of E-cadherin was correlated with depth of myometrial invasion and advanced stage of disease.[30,31] Type III carcinomas had a significantly reduced expression of E-cadherin compared to type I carcinomas and the expression even tended to

be lower than in type II carcinomas. This might explain a more aggressive behavior of type III carcinomas when compared to type I carcinomas.

KRAS mutations are described to be an early event in the carcinogenesis of endometrioid endometrial carcinoma and they were shown to be present in all endometrial hyperplasia or in atypical hyperplasia only.[28,32] These findings support the lack of *KRAS* mutations in endometrioid carcinomas arising from atrophic endometrium. In addition, Guerrero *et al*. have described up regulation of E-cadherin expression by *KRAS* mutations.[33] Subsequently, Singh *et al*. described that Zeb1, a transcription factor repressing E-cadherin expression, is expressed specifically in tumor cell lines that grow independent of *KRAS*.[34] These studies support our findings of a combination of few *KRAS* mutations and loss of E-cadherin in type III carcinomas. It is unclear whether loss of E-cadherin is an early event of carcinogenesis of type III carcinomas, or secondary to the fact that carcinogenesis of these carcinomas is less characterized by *KRAS* mutations. However, it is likely that loss of E-cadherin is (partly) responsible for previous findings that type III carcinomas are associated with adverse pathologic findings and an adverse outcome.[10]

This study is the first to analyze the immunohistochemical and genetic profile of endometrioid endometrial carcinomas with atrophic background endometrium. A large set of immunohistochemical markers and genes was analyzed to give a clear view of the similarities and differences between the different endometrial tumor types. A weakness may be the fact that DNA was extracted for mutation analysis from whole slides instead of selected tumor tissue. However, while this might be responsible for the high mutation rate we found in type II carcinomas, it does not interfere with answering the question whether there is a difference between type I and III carcinomas. If anything, it highlights more clearly that very few *KRAS* mutations are found in type III carcinomas as well as in the surrounding tissue.

In conclusion, on an immunohistochemical and genetic level, endometrioid carcinomas arising from atrophic background endometrium were shown to be quite comparable to endometrioid carcinomas arising from hyperplastic background endometrium. However, while *KRAS* mutations are an early event in carcinogenesis of endometrioid endometrial carcinoma in hyperplastic endometrium, such mutations were rare in endometrioid carcinomas with atrophic background endometrium. Carcinogenesis of these carcinomas

seems to be characterized by early loss of E-cadherin, which was previously associated with a worse prognosis.

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012;62(1):10-29.
2. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecologic oncology. 1983;15(1):10-7.
3. Liu FS. Molecular carcinogenesis of endometrial cancer. Taiwanese journal of obstetrics & gynecology. 2007;46(1):26-32.
4. Ellenson HL, Ronnett BM, Kurman RJ. Precursor lesions of endometrial carcinoma. In: Kurman RJ, Ellenson HL, Ronnett BM, editors. Blaustein's Pathology of the Female Genital Tract. Sixth ed. New York: Springer; 2011. p. 359-91.
5. Ellenson HL, Ronnett BM, Soslow RA, Zaino RJ, Kurman RJ. Endometrial carcinoma. In: Kurman RJ, Ronnett BM, Ellenson HL, editors. Blaustein's Pathology of the Female Genital Tract. Sixth ed. New York: Springer; 2011. p. 393-452.
6. Ellis PE, Ghaem-Maghami S. Molecular characteristics and risk factors in endometrial cancer: what are the treatment and preventative strategies? International journal of gynecological cancer : official journal of the International Gynecological Cancer Society. 2010;20(7):1207-16.
7. Engelsen IB, Akslen LA, Salvesen HB. Biologic markers in endometrial cancer treatment. APMIS. 2009;117(10):693-707.
8. Matias-Guiu X, Prat J. Molecular pathology of endometrial carcinoma. Histopathology. 2013;62(1):111-23.
9. Lax SF. Molecular genetic pathways in various types of endometrial carcinoma: from a phenotypical to a molecular-based classification. Virchows Archiv : an international journal of pathology. 2004;444(3):213-23.
10. Geels YP, Pijnenborg JM, van den Berg-van Erp SH, Bulten J, Visscher DW, Dowdy SC, et al. Endometrioid endometrial carcinoma with atrophic endometrium and poor prognosis. Obstetrics and gynecology. 2012;120(5):1124-31.
11. Roelofsen T, Geels YP, Pijnenborg JM, van Ham MA, Zomer SF, van Tilburg JM, et al. Cervical Cytology in Serous and Endometrioid Endometrial Cancer. International journal of gynecological pathology : official journal of the International Society of Gynecological Pathologists. 2013.
12. Roelofsen T, van Ham MA, Wiersma van Tilburg JM, Zomer SF, Bol M, Massuger LF, et al. Pure compared with mixed serous endometrial carcinoma: two different entities? Obstetrics and gynecology. 2012;120(6):1371-81.
13. Mills AM, Lonacre TA. Endometrial hyperplasia. Seminars in Diagnostic Pathology. 2010;27:199-214.
14. Kurman RJ, Kaminski PF, Norris HJ. The behavior of endometrial hyperplasia. A long-term study of "untreated" hyperplasia in 170 patients. Cancer. 1985;56(2):403-12.
15. McCluggage WG. Benign Diseases of the Endometrium. In: Kurman RJ, Ellenson LH, Ronnett BM, editors. Blaustein's Pathology of the Female Genital Tract. 6th ed: Springer; 2011. p. 305-58.
16. Fader AN, Starks D, Gehrig PA, Secord AA, Frasure HE, O'Malley DM, et al. An updated clinicopathologic study of early-stage uterine papillary serous carcinoma (UPSC). Gynecologic oncology. 2009;115(2):244-8.

17. Arafa M, Boniver J, Delvenne P. Progression model tissue microarray (TMA) for the study of uterine carcinomas. Disease markers. 2010;28(5):267-72.
18. Ioffe OB, Papadimitriou JC, Drachenberg CB. Correlation of proliferation indices, apoptosis, and related oncogene expression (bcl-2 and c-erbB-2) and p53 in proliferative, hyperplastic, and malignant endometrium. Human pathology. 1998;29(10):1150-9.
19. Huszar M, Pfeifer M, Schirmer U, Kiefel H, Konecny GE, Ben-Arie A, et al. Up-regulation of L1CAM is linked to loss of hormone receptors and E-cadherin in aggressive subtypes of endometrial carcinomas. The Journal of pathology. 2010;220(5):551-61.
20. Leake R, Barnes D, Pinder S, Ellis I, Anderson L, Anderson T, et al. Immunohistochemical detection of steroid receptors in breast cancer: a working protocol. UK Receptor Group, UK NEQAS, The Scottish Breast Cancer Pathology Group, and The Receptor and Biomarker Study Group of the EORTC. Journal of clinical pathology. 2000;53(8):634-5.
21. Lurkin I, Stoehr R, Hurst CD, van Tilborg AA, Knowles MA, Hartmann A, et al. Two multiplex assays that simultaneously identify 22 possible mutation sites in the KRAS, BRAF, NRAS and PIK3CA genes. PloS one. 2010;5(1):e8802.
22. Kompier LC, Lurkin I, van der Aa MN, van Rhijn BW, van der Kwast TH, Zwarthoff EC. FGFR3, HRAS, KRAS, NRAS and PIK3CA mutations in bladder cancer and their potential as biomarkers for surveillance and therapy. PloS one. 2010;5(11):e13821.
23. Moreno-Bueno G, Sanchez-Estevez C, Palacios J, Hardisson D, Shiozawa T. Low frequency of BRAF mutations in endometrial and in cervical carcinomas. Clin Cancer Res. 2006;12(12):3865; author reply -6.
24. Catasus L, D'Angelo E, Pons C, Espinosa I, Prat J. Expression profiling of 22 genes involved in the PI3K-AKT pathway identifies two subgroups of high-grade endometrial carcinomas with different molecular alterations. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc. 2010;23(5):694-702.
25. Catasus L, Gallardo A, Cuatrecasas M, Prat J. PIK3CA mutations in the kinase domain (exon 20) of uterine endometrial adenocarcinomas are associated with adverse prognostic parameters. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc. 2008;21(2):131-9.
26. Hayes MP, Wang H, Espinal-Witter R, Douglas W, Solomon GJ, Baker SJ, et al. PIK3CA and PTEN mutations in uterine endometrioid carcinoma and complex atypical hyperplasia. Clin Cancer Res. 2006;12(20 Pt 1):5932-5.
27. Kuhn E, Wu R-C, Guan B, Wu G, Zhang J, Wang Y, et al. Identification of molecular pathway aberrations in uterine serous carcinoma by genome-wide analyses. Journal of the National Cancer Institute. 2012;104(19):1503-13.
28. Sasaki H, Nishii H, Takahashi H, Tada A, Furusato M, Terashima Y, et al. Mutation of the Ki-ras protooncogene in human endometrial hyperplasia and carcinoma. Cancer Res. 1993;53(8):1906-10.
29. Takeichi M. Cadherin cell adhesion receptors as a morphogenetic regulator. Science. 1991;251(5000):1451-5.

30. Saito T, Nishimura M, Yamasaki H, Kudo R. Hypermethylation in promoter region of E-cadherin gene is associated with tumor dedifferention and myometrial invasion in endometrial carcinoma. Cancer. 2003;97(4):1002-9.
31. Moreno-Bueno G, Hardisson D, Sarrio D, Sanchez C, Cassia R, Prat J, et al. Abnormalities of E- and P-cadherin and catenin (beta-, gamma-catenin, and p120ctn) expression in endometrial cancer and endometrial atypical hyperplasia. The Journal of pathology. 2003;199(4):471-8.
32. Enomoto T, Inoue M, Perantoni AO, Buzard GS, Miki H, Tanizawa O, et al. K-ras activation in premalignant and malignant epithelial lesions of the human uterus. Cancer Res. 1991;51(19):5308-14.
33. Guerrero S, Casanova I, Farre L, Mazo A, Capella G, Mangues R. K-ras codon 12 mutation induces higher level of resistance to apoptosis and predisposition to anchorage-independent growth than codon 13 mutation or proto-oncogene overexpression. Cancer Res. 2000;60(23):6750-6.
34. Singh A, Greninger P, Rhodes D, Koopman L, Violette S, Bardeesy N, et al. A gene expression signature associated with “K-Ras addiction” reveals regulators of EMT and tumor cell survival. Cancer cell. 2009;15(6):489-500.

CHAPTER 7

COMPARISON OF A PANEL OF IMMUNOHISTOCHEMICAL MARKERS IN ENDOMETRIOID ENDOMETRIAL CARCINOMA WITH AND WITHOUT METASTATIC DISEASE

Y.P. Geels[1], A.A.G. van Tilborg[1,2], B.E.C. Nienhaus[1], S.H. van den Berg- van Erp[3], M.P.L.M. Snijders[4], L.F.A.G. Massuger[1], A. van der Wurff[5], J. Bulten[2], J.M.A. Pijnenborg[6]

[1] Department of Obstetrics and Gynecology, Radboud University Nijmegen, Medical Centre
[2] Department of Pathology, Radboud University Nijmegen, Medical Centre
[3] Department of Pathology, Canisius-Wilhelmina Hospital, Nijmegen
[4] Department of Obstetrics and Gynecology, Canisius-Wilhelmina Hospital, Nijmegen
[5] Department of Pathology, St. Elisabeth Hospital, Tilburg
[6] Department of Obstetrics and Gynecology, TweeSteden Hospital, Tilburg
The Netherlands

Submitted

Abstract

Introduction: The aim of this study was to analyze differences in immunohistochemical expression patterns in endometrioid endometrial carcinomas (EECs) with and without metastases. Second, the aim was to look for potential differences in immunohistochemical profile between the primary and the metastatic tumor.

Methods: All patients with FIGO stage III or IV EEC treated in four hospitals in the south of the Netherlands between 1999 and 2010 were selected as cases. A control group treated for FIGO stage I disease in two of these four hospitals was matched on tumor grade. Tissue Micro Arrays (TMAs) were prepared, and slides of the TMAs were stained with p53, p16, p21, MIB1, β-catenin, E-cadherin, MLH1, PMS2, ER, PR, L1CAM, and PTEN.

Results: Significant lower ER expression was found in the primary tumor of patients with metastases compared to patients without metastases (P=0.04). Significant stronger p16 and p21 expression was found when tumor tissue from the metastatic site was compared to the primary tumor (P=0.01 and P=0.02 respectively). In addition, when comparing the primary tumor tissue of patients with locoregional and distant metastases, the distant metastasizing tumors showed significant lower ER and PR expression, and increased MIB1 expression (P=0.04, P=0.04, and P<0.01 respectively).

Conclusion: In conclusion, this study gives more insight into the expression patterns of immunohistochemical markers related to the metastatic potential of EECs. ER, PR, p16, p21, and MIB1 may be involved in the metastatic process of EECs.

Introduction

Endometrial carcinoma has become the most common gynecologic cancer in the western world today, counting for 1900 newly diagnosed patients annually in the Netherlands.[1,2] This increasing incidence can be partly attributed to increasing obesity and life expectancy.[3,4] Endometrial carcinoma can be divided into two subtypes with different clinical and pathologic characteristics.[5] Type I carcinomas occur around the age of 60, have a good prognosis, and are directly related to unopposed estrogen stimulation. The majority of these carcinomas arise in a background of hyperplastic endometrium and show endometrioid histology. Type II, non-endometrioid carcinomas occur in women around the age of 70, are unrelated to estrogen stimulation, arise in atrophic endometrium, and have a relatively poor prognosis.[6] Several molecular pathways are known to be involved in the carcinogenesis of endometrial carcinoma. Type I carcinomas show microsatellite instability, and mutations in *PTEN, PIK3CA, KRAS* and *CTNNB1* (β-catenin), whereas type II carcinomas reveal alterations in *TP53*, and molecular alterations of *STK15, CDKN2A* (p16)*, CDH1* (E-cadherin) and *ERBB2* (Her2/neu).[7,8] Although the prognosis of endometrioid endometrial carcinoma (EEC) in general is favorable, a minority of these carcinomas do metastasize. Little is known about the molecular pathways responsible for the process of metastasizing in endometrioid endometrial cancer. The role of epithelial to mesenchymal transition and the loss of E-cadherin expression in endometrioid endometrial carcinoma have been described recently.[9] Furthermore, changed expression of ER, PR, Stathmin, and p-mTOR was found in recurrent endometrial carcinoma when compared to the primary tumor.[10] In addition, differential expression patterns of p21, p53, and MIB were associated with poor prognosis in endometrial carcinoma. Changes in the *MLH1* gene were also found to play a role in the carcinogenesis of non-hereditary EECs.[8,11,12] L1CAM has been recently reported as an important immunohistochemical marker with superior prognostic properties in EEC.[13]

The cornerstone of the treatment of endometrial carcinoma is surgery. In the Netherlands, adjuvant radiotherapy is advised when two out of three risk factors, age > 60, myometrial invasion > 50%, and tumor grade 3, are present.[14] The treatment strategy of type I carcinomas differs from that of type II carcinomas. To date, in The Netherlands, for type II carcinomas a complete surgical staging procedure is advised. In addition, adjuvant chemotherapy may be considered. For type I, low grade EEC hysterectomy and bilateral salpingo-oophorectomy is

advised. For high grade endometrioid carcinoma a consensus about the optimal treatment has not yet been conceived in the Dutch guidelines.[2] A more thorough understanding of the mechanisms underlying the metastatic potential of EEC may provide routes to individualize treatment.

The aim of the present study was, primarily, to analyze potential differences in immunohistochemical expression patterns in endometrioid endometrial carcinomas with metastases compared to endometrioid endometrial carcinomas limited to the uterus. Secondary, the aim was to look for potential changes in immunohistochemical profile of the primary tumor and the metastatic site. To this end, a panel of markers known to be altered in aggressive endometrial carcinoma was composed.

Methods

Patient selection and histopathologic review

For this case-control study the Dutch nationwide network and registry of histology and cytopathology (Pathologisch Anatomisch Landelijk Geautomatiseerd Archief: PALGA) was used to search for all patients diagnosed and surgically treated for FIGO stage III or IV endometrioid endometrial carcinoma (EEC) at the Radboud University Nijmegen Medical Centre (RUNMC), the Canisius-Wilhelmina Hospital Nijmegen, the TweeSteden Hospital Tilburg, and the St. Elisabeth Hospital Tilburg, between January 1999 and January 2010. A total of 53 EEC patients with FIGO stage III or IV were identified.

All histologic slides of the primary carcinoma and the metastatic site were retrieved from the pathology archives and reviewed. Review was done systematically including the following items: the histologic type and tumor grade of both the primary tumor and the metastasis or metastases. Of the primary tumor site, depth of myometrial invasion, presence of lymphovascular space invasion, and the nature of the background endometrium were reviewed as well.[15] Review was performed in every hospital separately by an independent experienced pathologist (JB, SB, AW), who was unaware of the results of the original pathology reports or the clinical outcome of the patients. There was no systematic review between the centers. In case of doubt about the diagnosis, or in case of discrepancy with the original pathology report consensus between the three reviewing pathologists was achieved.

After review, in total 15 cases were excluded for several reasons. Ten patients were excluded because of a diagnosis of non-endometrioid histology like serous, clear cell, or undifferentiated endometrial carcinoma after review. One patient was excluded because the metastatic site was diagnosed as a second primary malignancy after review, and from four patients insufficient material was available. Thus, a total of 38 patients comprised our EEC case group; tumor grade 1 (N=3), tumor grade 2 (N=16), and tumor grade 3 (N=19).

Control group selection

Subsequently, a matched control group was collected from a well defined, reviewed database with endometrial cancer patients diagnosed in the RUNMC and the Canisius-Wilhelmina Hospital from January 1999 until January 2010.[16] The control group consisted of clinical FIGO stage I EEC patients and was matched on tumor grade. A total of 37 EEC patients comprised the control group: tumor grade 1 (N=4), tumor grade 2 (N=17), and tumor grade 3 (N=16).

Patient characteristics

Clinical data were abstracted from patient records. Age, menopausal state, body mass index (BMI), parity, medical history, treatment, stage of disease, date of recurrence of disease, date of death, and the cause of death were registered. Stage of disease was based on the 2009 International Federation of Gynecology and Obstetrics (FIGO) staging system.[17] Follow up data were extracted from the medical charts. The median time of follow up was 36 months (range 1-105).

Tissue microarray and immunohistochemistry (IHC)

Tissue microarrays were created from the primary carcinoma, the corresponding metastatic site, and from the carcinomas in the control group. A representative area of the carcinoma was selected on hematoxylin and eosin-stained slides. One cylinder with a diameter of 3 mm was punched out the donor block from the selected area, and mounted into a recipient paraffin block using a manual tissue microarray (Tissue-Tek, Quick-Ray, Sakura Finetek, USA). The tissue microarrays were cut in 4 µm slides and immunohistochemically stained. Briefly, immunohistochemical analysis for p53, p16, p21, MIB1, β-catenin, E-cadherin, MLH1, PMS2, ER and PR expression was performed as follows: after EDTA antigen retrieval, sections were

stained with the primary antibody using the automated I6000 immunostainer (Biogenics, San Ramos, California, USA). Staining of tissue was visualized using 3,3'-Diaminobenzidine (Zymed lab, California, USA) as substrate and counterstained with Mayer's haematoxylin.
For PTEN and L1CAM staining formalin fixed paraffin sections were deparaffinized and rehydrated. EDTA antigen retrieval was performed and staining was done on a Manual Staining Box lined with moist toweling. Endogenous background was blocked using Peroxidase Blocking Reagent (3%H2O2 in PBS). A protein blocking step was added using horse serum. The slides were incubated with PTEN or L1CAM overnight at 4°C. Subsequently a secondary horse anti-mouse antibody was added. Visualization was performed with the Vectastain ABC Method (Vector Laboratories, CA, USA) and 3,3'-Diaminobenzidine (Zymed lab, California, USA) as substrate. Staining was enhanced in $CuSO_4$ and slides were counterstained with Mayer's haematoxylin. Finally, slides were dehydrated and mounted. An overview of all antibodies used in this study is shown in Table 1.

Evaluation of staining

Two authors independently evaluated the stained slides semi quantitatively by standard light microscopy (YG, HB). They were unaware if the tissue cylinders were primary, metastatic, or from the control group. Categories were made for the percentage of stained nuclei: 0

Table 1: Antibodies used for staining.

Antibody	Clone	Vendor	Retrieval	Dilution	Staining
β-catenin	14/Beta-catenin	BD	EDTA 10'	1:100	Membranous, cytoplasm
E-cadherin	SPM471	Thermo Scientific	EDTA 10'	1:300	Membranous
ER	SP1	Thermo Scientific	EDTA 10'	1:80	Nuclear
PR	PgR636	DAKO	citrate 10'	1:250	Nuclear
MLH1	G168-15	BD	EDTA 10'	1:50	Nuclear
PMS2	A16-4	BD	EDTA 10'	1:100	Nuclear
L1CAM	UJ127	Thermo Scientific	EDTA 10'	1:100	Membranous
MIB	MIB-1	DAKO	citrate 10'	1:100	Nuclear
PTEN	6H2.1	DAKO	EDTA 10'	1:100	Nuclear, cytoplasm
P53	DO-7	Thermo Scientific	citrate 10'	1:400	Nuclear
P16	G175-405	BD	citrate 10'	1:20	Nuclear, cytoplasm
P21	CDS-60.2	Neomarkers	citrate 10'	1:75	Nuclear

(0% of the tumor cells positive), 1 (0-10% of the tumor cells positive), 2 (10-50% of the tumor cells positive), and 3 (>50% of the tumor cells positive). The intensity of the staining was subdivided in 0 (none), 1 (weak), 2 (moderate), and 3 (strong). A staining index was calculated as the product of nuclear staining intensity and staining area (range 0-9).[10]

Statistical analysis

Comparison was made between the primary carcinoma of the case group and the primary carcinoma of the control group. Differences in clinical and pathologic parameters were tested for statistical significance using the Pearson's Chi-square (χ^2) test or the Fisher's exact test, and the Mann-Whitney test. Comparison of the IHC expression was made between the primary carcinoma of the case-group and the primary carcinoma of the control group, and between the primary carcinoma of the case group and the metastatic site of the case group. In addition, a comparison of IHC expression was made between patients with loco-regional metastases (direct local invasion and spread through the abdominal cavity) and distant metastases (lymphogenous and hematogenous spread). Boxplots were generated to depict the differences in IHC expression, and statistical significance was tested using the Mann-Whitney test. The *P*-values presented are two-sided, and associations were considered significant if the *P*-value was less than 0.05. Statistical analyses were performed using the software package SPSS 20.0 for Microsoft Windows (SPSS Inc).

Results

Clinical and pathologic characteristics

The clinical and pathologic characteristics of all patients are shown in Table 2. A comparison of the distribution and presence of clinicopathologic characteristics was made between the group with and the group without metastatic disease. Median age, BMI and menopausal status were similar between the two groups. Since the group with metastases had advanced FIGO stage and the control group had clinical FIGO stage I disease, more patients were treated with adjuvant radiotherapy and/or chemotherapy in the group with metastases. Also, more patients had deep myometrial invasion, cervical involvement and lymphovascular space invasion. In addition, in the metastatic group, more patients died as a consequence of the carcinoma. Median follow-up was longer in the group without metastatic disease.

Table 2: Clinical and pathologic characteristics of all patients (N=75).

	Patient group (N=38)		Control group (N=37)		*P*-value
Variable	N / median	% / range	N / median	% / range	
Median age (years)	65.5	49-82	66.0	45-82	0.84
Median Body Mass Index (kg/m^2)	28.7	19.4-50.8	28.3	18.7-41.4	0.87
Menopausal state					0.96
Premenopausal	5	13.0	5	13.5	
Postmenopausal	33	87.0	32	86.5	
FIGO stage (2009)					
IA	0	0	26	70.3	
IB	0	0	11	29.7	
II	0	0	0	0	
III	19	50.0	0	0	
IV	19	50.0	0	0	
Adjuvant radiotherapy					0.04
No	11	28.9	22	59.5	
Yes, external beam therapy	10	26.3	9	24.3	
Yes, vaginal brachy therapy	0	0	6	16.2	
Yes, combination	11	28.9	0	0	
Unknown	6	15.8	0	0	
Adjuvant chemotherapy					0.02
No	31	81.6	37	100	
Yes	5	13.2	0	0	
Unknown	2	5.3	0	0	
Grade					1.00
1	3	7.9	4	10.8	
2	16	42.1	17	45.9	
3	19	50.0	16	43.2	
Myometrial invasion					<0.01
<1/2	7	18.4	22	61.1	
≥1/2	31	81.6	14	38.9	
Cervical involvement					<0.01
No	28	55.3	35	94.6	
Endocervical	7	18.4	2	5.4	
Stromal	10	26.3	0	0.0	
Lymphovascular space invasion					<0.01
No	15	39.5	34	91.9	
Yes	23	60.5	3	8.1	
Recurrence					<0.01
No	14	36.8	37	100	
Yes, local	2	5.3	0	0	
Yes, regional	4	10.5	0	0	
Yes, distance	14	36.8	0	0	
Unknown	4	10.5	0	0	
Patient died					
No	26	68.4	36	97.3	<0.01
Yes, consequence of disease	12	31.6	0	0	
Yes, other cause	0	0	1	2.7	
Time of follow up (months)	27	1-105	44	1-79	0.01

All cases with metastatic disease were FIGO stage III or IV. The localization of the most distant metastatic sites of these cases are shown in Table 3. Of the 38 patients with metastatic disease, the most distant metastatic site was local in 17 patients (44.7%), the abdominal cavity in seven patients (18.4%), lymphogenous in ten patients (26.3%), and hematogenous (liver/lung) metastases in four patients (10.6%). One of the four patients with hematogenous metastases had proven lymphatic spread of disease as well.

Immunohistochemical expression

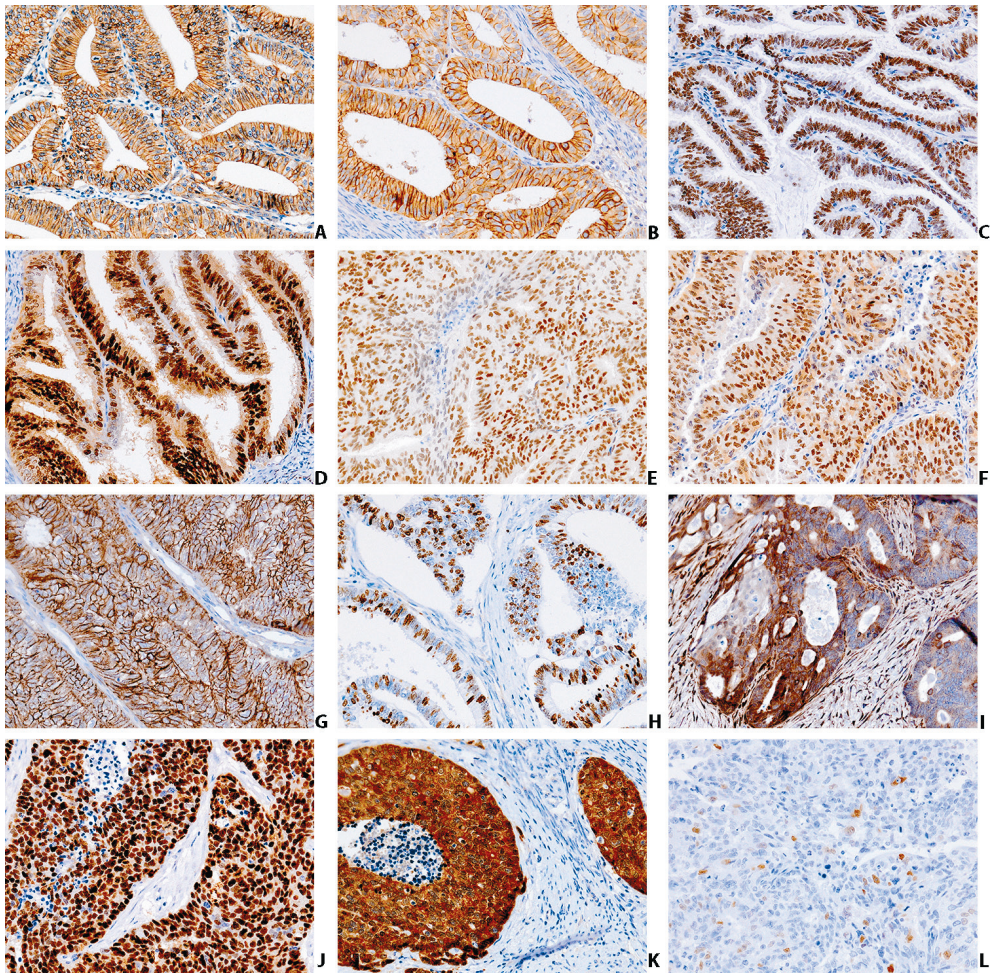
Examples of immunohistochemical expression patterns of all antibodies are presented in Figure 1. An overview of the numbers of patients with negative or positive staining is given in Table 4. The tumor was considered negative with a staining index of 0-1, and the tumor was considered positive with a staining index of 2-9.

Differences in immunohistochemical expression between the primary tumor of patients with metastatic disease and the primary tumor of patients in the control group are depicted in Figure 2. When comparing the group with metastatic disease with the control group, apart from a significant loss of ER expression in the group with metastatic disease (*P*=0.04),

Table 3: Sites of metastases in the cases with metastatic disease.

	Site	N	Total
Locoregional	Ovaries and/or tubes	4	
	Parametrium	2	
	Vagina	4	
	Peritoneum bladder	1	
	Sigmoid, descendent colon, distal ileum, cecum, appendix	5	
	Sacro-uterine ligament	1	17 (44.7%)
Abdominal	Omentum	3	
	Peritoneum of the abdominal cavity	3	
	Diaphragm	1	7 (18.4%)
Lymphogenous	Pelvic	6	
	Para-aortal lymph nodes	3	
	Inguinal lymph node	1	10 (26.3%)
Hematogenous	Liver	2	
	Lung	2	4 (10.5%)

Figure 1: Examples of positive IHC staining.



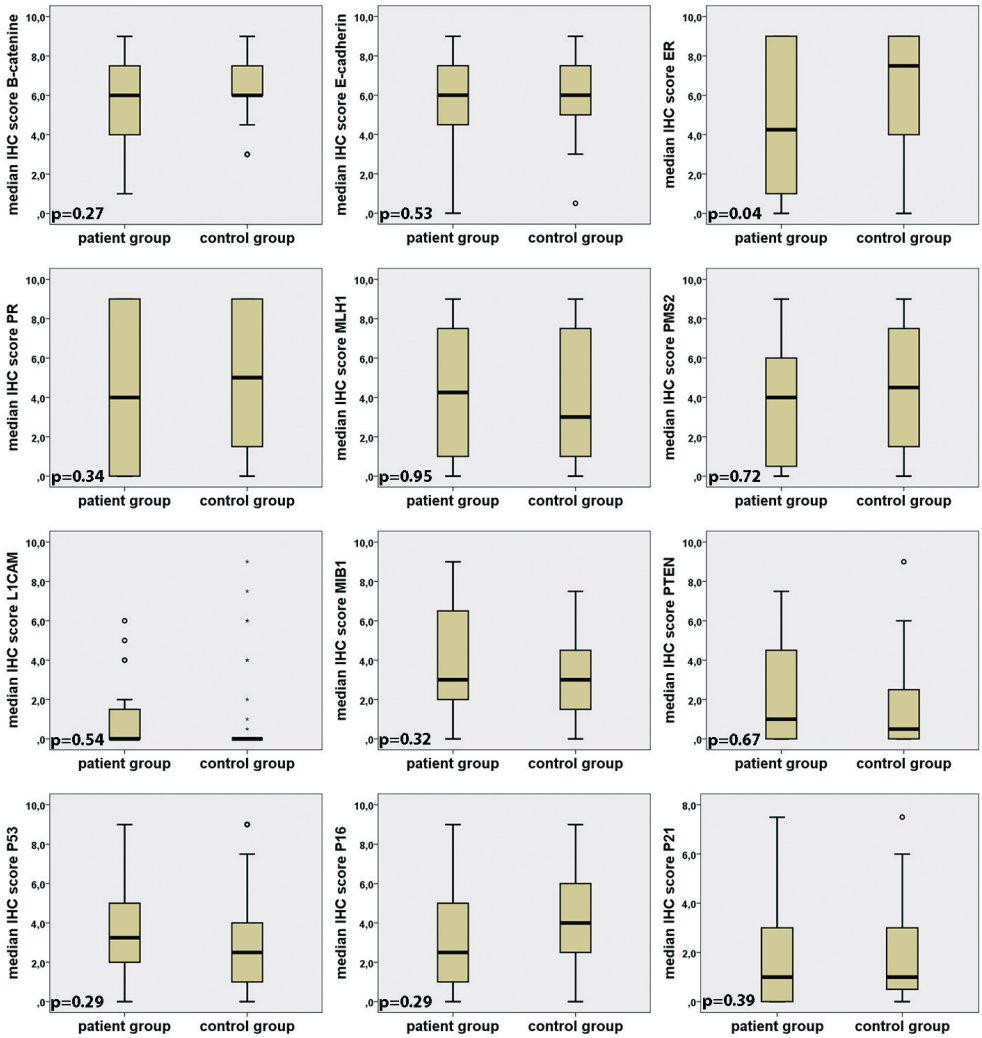
β-catenine (A), E-cadherin (B), ER (C), PR (D), MLH1 (E), PMS2 (F), L1CAM (G), MIB1 (H), PTEN (I), p53 (J), p16 (K), and p21 (L).

Table 4: Comparison between primary tumor of patients with and without metastases, the primary tumor and the metastatic site, and locoregional and distant metastases.

	Cases N (%)	Control N (%)	Primary N (%)	Metastasis N (%)	Locoregional N (%)	Distant N (%)
Bcat						
Neg*	1 (2.6)	0 (0.0)	1 (2.6)	2 (6.3)	1 (4.2)	0 (0.0)
Pos	37 (97.4)	37 (100.0)	37 (97.4)	30 (93.8)	23 (95.8)	14 (100.0)
Ecad						
Neg	3 (7.9)	1 (2.7)	3 (7.9)	6 (18.8)	1 (4.2)	2 (14.3)
Pos	35 (92.1)	36 (97.3)	35 (92.1)	26 (81.3)	23 (95.8)	12 (85.7)
ER						
Neg	10 (26.3)	2 (5.4)	10 (26.3)	6 (20.0)	3 (12.5)	7 (50.0)
Pos	28 (73.7)	35 (94.6)	28 (73.3)	24 (80.0)	21 (87.5)	7 (50.0)
PR						
Neg	13 (34.2)	9 (24.3)	13 (34.2)	18 (54.5)	5 (20.8)	8 (57.1)
Pos	25 (65.8)	28 (75.7)	25 (65.8)	15 (45.5)	19 (79.2)	6 (42.9)
MLH1						
Neg	11 (28.9)	11 (29.7)	11 (28.9)	6 (18.8)	9 (37.5)	2 (14.3)
Pos	27 (71.1)	26 (70.3)	27 (71.1)	26 (81.3)	15 (62.5)	12 (85.7)
PMS2						
Neg	11 (28.9)	8 (21.6)	11 (28.9)	8 (25.0)	8 (33.3)	3 (21.4)
Pos	27 (71.1)	29 (78.4)	27 (71.1)	24 (75.0)	16 (67.7)	11 (78.6)
L1CAM						
Neg	26 (68.4)	29 (78.4)	27 (71.1)	20 (66.7)	17 (70.8)	10 (71.4)
Pos	10 (26.3)	7 (18.9)	11 (28.9)	10 (33.3)	7 (29.2)	4 (28.6)
MIB						
Neg	6 (15.8)	6 (16.2)	6 (15.8)	5 (16.1)	6 (25)	0 (0.0)
Pos	32 (84.2)	31 (83.8)	32 (84.2)	26 (83.9)	18 (75)	14 (100.0)
PTEN						
Neg	21 (55.3)	20 (54.1)	21 (55.3)	10 (33.3)	12 (50.0)	9 (69.2)
Pos	16 (42.1)	17 (45.9)	16 (42.1)	20 (66.7)	12 (50.0)	4 (30.8)
P53						
Neg	5 (13.2)	10 (27.0)	5 (13.2)	0 (0.0)	4 (16.7)	1 (7.1)
Pos	33 (86.8)	27 (73.0)	33 (86.3)	32 (100.0)	20 (83.3)	13 (92.9)
P16						
Neg	10 (26.3)	4 (10.8)	10 (26.3)	3 (9.1)	7 (29.2)	3 (21.4)
Pos	28 (73.7)	33 (89.2)	28 (73.3)	30 (90.9)	17 (70.7)	11 (78.6)
P21						
Neg	21 (55.3)	19 (51.4)	21 (55.3)	7 (22.6)	13 (54.2)	8 (57.1)
Pos	17 (44.7)	18 (48.6)	17 (44.7)	24 (77.4)	11 (45.8)	6 (42.9)

* A staining index of 0-1 was considered negative, and a staining index of 2-9 was considered positive.

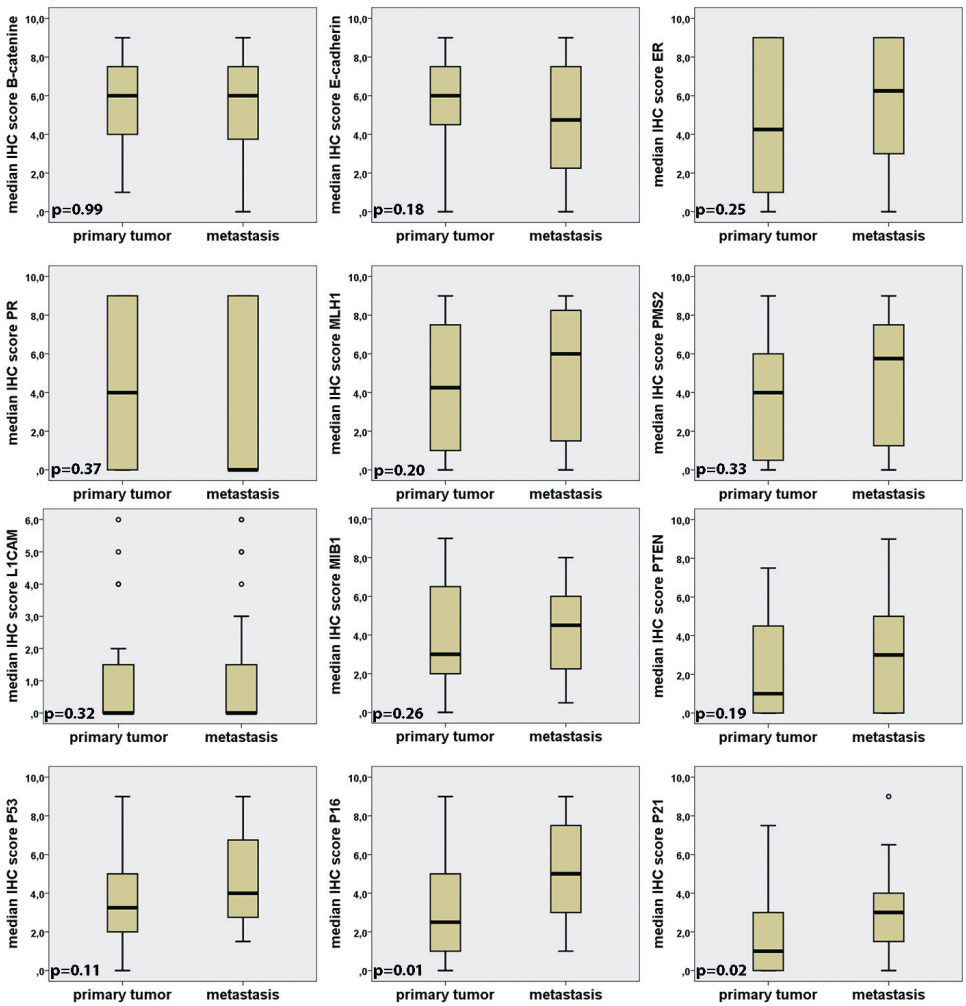
Figure 2: IHC staining score of the primary tumor of patient with metastases, compared to the primary tumor of patients without metastases depicted with boxplots.



P-values were calculated using the Mann-Whitney Test.

no significant differences in staining patterns in the primary tumor were observed. Figure 3 shows the difference in expression among the patients with metastatic disease between the primary and the metastatic tumor. In this immunohistochemical comparison, the metastatic sites showed significant more p16 and p21 expression compared to the primary tumor (*P*=0.01 and *P*=0.02 respectively).

Figure 3: IHC staining score of the primary tumor site, compared to the metastatic site within the same patient depicted with boxplots.

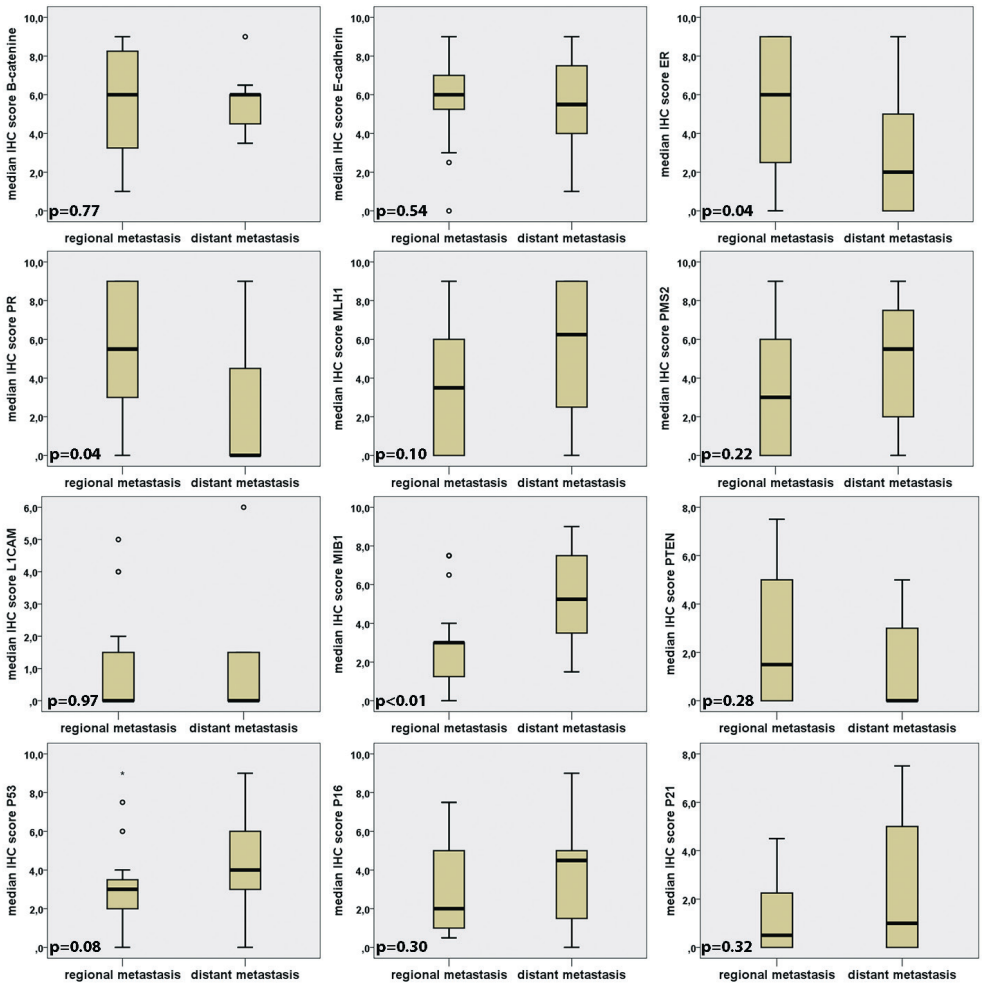


P-values were calculated using the Mann-Whitney Test.

Differences in staining pattern between different sites of metastases

In Figure 4, the patients with locoregional and/or abdominal spread (N=24) were grouped and compared with respect to the immunohistochemical expression pattern of the primary tumor with the primary tumor of patients with lymphogenous and/or hematogenous spread of disease (n=14). In the group with lymphogenous and hematogenous spread

Figure 4: IHC staining score of the primary tumor of patients with loco-regional metastases compared to the primary tumor of patients with distant metastases depicted with boxplots.



P-values were calculated using the Mann-Whitney Test.

there was more expression of MIB, and less expression of ER and PR (*P*=<0.01, 0.04, and 0.04 respectively). When we compared the primary tumor of patients with lymphogenous metastases (N=10) with the primary tumor of patients with hematogenous metastases (N=4) no significant differences in expression of the immunohistochemical panel were found (data not shown).

Discussion

In this study, immunohistochemical profiles in EEC patients with and without metastatic disease were compared. Low or absent ER expression was found significantly more frequently in the primary tumor of patients with metastatic EEC. In addition, we compared the primary and the metastatic tumor, and found an increase of p21 and p16 expression in the metastatic site. Furthermore, the immunohistochemical profile of local and distant metastases demonstrated differences in the expression of ER, PR, and MIB.

Loss of ER expression in EEC related with a more aggressive clinical course is already known for many years. ER and PR are independent prognostic factors for survival and recurrence.[7,8] Loss of ER expression has also been reported in recurrent tumors of endometrial carcinoma patients.[10] As mentioned before, type I carcinomas are directly related to estrogen. Estrogen and progesterone, binding to estrogen receptors (ER) and progesterone receptors (PR) respectively, lead to specific phenotypic effects.[4] Estrogen promotes cell proliferation and inhibits apoptosis, including modulation of tumor suppressor function.[18] In tumor grade 2 and 3 EECs, it has been demonstrated that increased expression of ER and PR is associated with a better prognosis.[19,20]

The results of this study show a significant decrease in ER expression in the primary tumor of EEC patients with metastatic disease when compared to patients with the carcinoma confined to the uterus, thus more or less predicting the presence of metastases. Possibly, the carcinogenesis of these metastatic EECs differs from the estrogen stimulated carcinogenesis of type I carcinomas. We previously suggested the existence of an estrogen independent carcinogenetic pathway in EEC patients.[21] Furthermore, ER and PR expression were significantly more decreased in patients with distant hematogenous and lymphogenous spread of disease. This emphasizes the predictive value of ER and PR in EEC. It furthermore underlines that loss of ER and PR has an important role in tumor progression in EEC. ER and PR would be a useful help in the preoperative setting for therapy decision making.

MIB-1 is a cell-cycle regulator with increased expression in about half of endometrial carcinomas, associated with poor clinical outcome, vascular invasion, and hormone receptor loss.[8,22] In the current study, the expression of MIB-1 was found more frequently in the primary tumor of patients with lymphogenous and hematogenous metastases when compared to the primary tumor of patients with abdominal and loco-regional metastases.

This indicates that activation of this proliferation marker may be one of the steps in the process of distant metastasizing. High MIB1 expression could therefore be a useful marker for the identification of EEC with potential aggressive behavior.

Low expression-patterns of p16 have been associated with poor prognosis in endometrial carcinoma.[23-26] However, increased p16 expression was associated with high FIGO stage in tumor grade 3 EECs.[27] Over-expression of p16 has been observed at the invasive front of endometrial carcinomas.[28] In the current study, the metastatic site shows an increase of p16 expression when compared to the primary tumor. In literature, a possible explanation for these conflicting data can be found. There are three mechanisms known that alter the *P16* gene, i.e. homozygous deletion, promoter hypermethylation, and point mutations, all giving different degrees of aberrant protein staining.[23,29,30] A substantial part of the underlying mechanism of *P16* inactivation in endometrial carcinoma is not known. Loss of expression has been reported in 14-74% of endometrial carcinomas, whereas mutations, deletions and promoter methylation only occur in 2-6% of the endometrial carcinomas.[8]

In addition, conflicting data were found for p21 expression, a critical downstream effector in the P53 pathway, inducing cell cycle growth arrest.[31] The inactivation of *P21* could lead to tumor progression.[11] In literature, a loss of expression was associated with poor prognosis in endometrial carcinoma.[11,26,32] However, a gradual increase of expression of MIB-1, p16, and p21, from inactive endometrium to hyperplasia to EEC has also been reported.[33] In the current study, a gain of p21 expression was found in the metastatic tumor when compared to the primary tumor. It is known from literature that a mutation of the *P53* tumor-suppressor gene leads to accumulation of the mutant protein, showing increased expression of nuclear p53.[18,19,34] This means that immunohistochemical expression of the protein is not equivalent to a functional protein status. Possibly, the same mechanism applies for mutation of the *P21* gene, resulting in over expression of the mutant protein in the metastatic site.

The abovementioned studies reported on p16 and p21 expression in the primary tumor,[11,23-26-32] whereas in the current study we analyzed both primary and metastatic tumors. It may be possible that alterations in the *P16* and *P21* gene mark a next step in tumor progression of EECs. As mentioned before, a gradual increase of p16 and p21 expression was found comparing hyperplasia to inactive endometrium, and EEC to hyperplasia.[28] The current study follows this line by finding an increase of p16 and p21 expression when comparing the metastatic tumor to the primary tumor in EEC patients.

In the current study, some immunohistochemical markers known to be predictors of poor survival, such as p53, L1CAM and E-cadherin did not reveal a significantly different expression pattern between the metastatic and control group, neither in the metastatic group between the primary and the metastatic tumor.[8,12,13] P53 did show a trend of increased expression in 1) the primary tumor of patients with metastasis in comparison to the control group, 2) the metastatic site when compared to the primary tumor, and 3) the distant metastases when compared to loco-regional metastases. Also E-cadherin showed a trend of decrease in expression in the metastatic site when compared to the primary tumor. L1CAM did not show any difference in all comparisons. This observation may be partially explained by the low prevalence of L1CAM expression in EEC patients, but also by the low staining intensity and percentage of positive cells in L1CAM positive patients. In literature, 18% of patients in a large EEC cohort were L1CAM positive [13]. In the current study, containing a selected group of metastatic EECs, 18 of the 75 patients (24%) showed L1CAM expression.

This is a unique study, analyzing a large set of immunohistochemical markers in EEC patients. The cohort of EEC patients with histologically proven metastatic disease is relatively large and the tissue of the metastatic site was available for immunohistochemical analysis. Besides, the control group was matched on tumor grade, avoiding this possible bias in the IHC comparison between patients with and without metastatic disease. The shortcoming of this study is that it is a retrospective study harboring potential selection bias. Furthermore, all patients were treated according to the standardized Dutch guidelines of endometrial carcinoma treatment.[2] This implicates that for the majority of the patients no routine lymphadenectomy i.e. formal staging was performed. Metastatic disease was surgically removed when there was a clear suspicion of metastatic disease during either pre-operative workup or during the operation. It further means that in only a minority of the patients in the control group the absence of lymphogeneous metastatic disease was histologically proven by a lymphadenectomy. However, no patient in the control group did develop recurrent disease during a median follow-up period of 44 months, which emphasizes the absence of metastatic disease at primary treatment.

In conclusion, this study gives more insight into the expression patterns of IHC markers potentially involved in the metastatic process of endometrioid endometrial carcinomas. Knowledge about these expression patterns may be useful in the pre-operative risk assessment of EEC patients and thus the individualization of treatment. More research

needs to be performed to gain more insight into the processes responsible for progression of disease in endometrioid endometrial carcinomas.

References

1. Amant F, Mirza MR, Creutzberg CL. Cancer of the corpus uteri. Int J Gynaecol Obstet. 2012;119 Suppl 2:S110-7. Epub 2012/10/04.
2. www.oncoline.nl. 2011.
3. Boll D, Verhoeven RH, van der Aa MA, Pauwels P, Karim-Kos HE, Coebergh JW, et al. Incidence and survival trends of uncommon corpus uteri malignancies in the Netherlands, 1989-2008. Int J Gynecol Cancer. 2012;22(4):599-606. Epub 2012/03/09.
4. Kitchener HC, Trimble EL, Endometrial Cancer Working Group of the Gynecologic Cancer I. Endometrial cancer state of the science meeting. Int J Gynecol Cancer. 2009;19(1):134-40. Epub 2009/03/05.
5. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol. 1983;15(1):10-7. Epub 1983/02/01.
6. Ellenson HL, Ronnett BM, Soslow RA, Zaino RJ, Kurman RJ. Endometrial carcinoma. In: Kurman RJ, Ronnett BM, Ellenson HL, editors. Blaustein's Pathology of the Female Genital Tract. Sixth ed. New York: Springer; 2011. p. 393-452.
7. Yeramian A, Moreno-Bueno G, Dolcet X, Catasus L, Abal M, Colas E, et al. Endometrial carcinoma: molecular alterations involved in tumor development and progression. Oncogene. 2012. Epub 2012/03/21.
8. Engelsen IB, Akslen LA, Salvesen HB. Biologic markers in endometrial cancer treatment. APMIS. 2009;117(10):693-707. Epub 2009/09/25.
9. Montserrat N, Mozos A, Llobet D, Dolcet X, Pons C, de Herreros AG, et al. Epithelial to mesenchymal transition in early stage endometrioid endometrial carcinoma. Hum Pathol. 2012;43(5):632-43. Epub 2011/09/24.
10. Vandenput I, Trovik J, Leunen K, Wik E, Stefansson I, Akslen L, et al. Evolution in endometrial cancer: evidence from an immunohistochemical study. Int J Gynecol Cancer. 2011;21(2):316-22. Epub 2011/07/08.
11. Salvesen HB, Iversen OE, Akslen LA. Prognostic significance of angiogenesis and Ki-67, p53, and p21 expression: a population-based endometrial carcinoma study. J Clin Oncol. 1999;17(5):1382-90. Epub 1999/05/20.
12. Prat J, Gallardo A, Cuatrecasas M, Catasus L. Endometrial carcinoma: pathology and genetics. Pathology. 2007;39(1):72-87. Epub 2007/03/17.
13. Zeimet AG, Reimer D, Huszar M, Winterhoff B, Puistola U, Abdel Azim S, et al. L1CAM in Early-Stage Type I Endometrial Cancer: Results of a Large Multicenter Evaluation. J Natl Cancer Inst. 2013. Epub 2013/06/20.
14. Creutzberg CL, van Putten WL, Koper PC, Lybeert ML, Jobsen JJ, Warlam-Rodenhuis CC, et al. Surgery and postoperative radiotherapy versus surgery alone for patients with stage-1 endometrial carcinoma: multicentre randomised trial. PORTEC Study Group. Post Operative Radiation Therapy in Endometrial Carcinoma. Lancet. 2000;355(9213):1404-11. Epub 2000/05/03.
15. Silverberg SG, Mutter GL, Kurman RJ, Kubik-Huch RA, Nogales F, Tavassoli FA. Tumors of the uterine corpus: epithelial tumors and related lesions. In: Tavassoli FA, Stratton FR, editors. WHO

classification of tumors: pathology and genetics of tumors of the breast and female genital organs: World Health Organisation; 2003. p. 221-32.

16. Geels YP, Pijnenborg JM, van den Berg-van Erp SH, Snijders MP, Bulten J, Massuger LF. Absolute depth of myometrial invasion in endometrial cancer is superior to the currently used cut-off value of 50%. Gynecol Oncol. 2013;129(2):285-91. Epub 2013/02/21.
17. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Int J Gynaecol Obstet. 2009;105(2):103-4. Epub 2009/04/16.
18. Hecht JL, Mutter GL. Molecular and pathologic aspects of endometrial carcinogenesis. J Clin Oncol. 2006;24(29):4783-91. Epub 2006/10/10.
19. Suthipintawong C, Wejaranayang C, Vipupinyo C. Prognostic significance of ER, PR, Ki67, c-erbB-2, and p53 in endometrial carcinoma. J Med Assoc Thai. 2008;91(12):1779-84. Epub 2009/01/13.
20. Oreskovic S, Babic D, Kalafatic D, Barisic D, Beketic-Oreskovic L. A significance of immunohistochemical determination of steroid receptors, cell proliferation factor Ki-67 and protein p53 in endometrial carcinoma. Gynecol Oncol. 2004;93(1):34-40. Epub 2004/03/30.
21. Geels YP, Pijnenborg JM, van den Berg-van Erp SH, Bulten J, Visscher DW, Dowdy SC, et al. Endometrioid endometrial carcinoma with atrophic endometrium and poor prognosis. Obstet Gynecol. 2012;120(5):1124-31. Epub 2012/10/24.
22. Engelsen IB, Mannelqvist M, Stefansson IM, Carter SL, Beroukhim R, Oyan AM, et al. Low BMI-1 expression is associated with an activated BMI-1-driven signature, vascular invasion, and hormone receptor loss in endometrial carcinoma. British journal of cancer. 2008;98(10):1662-9. Epub 2008/05/14.
23. Ignatov A, Bischoff J, Schwarzenau C, Krebs T, Kuester D, Herrmann K, et al. P16 alterations increase the metastatic potential of endometrial carcinoma. Gynecol Oncol. 2008;111(2):365-71. Epub 2008/09/16.
24. Salvesen HB, Das S, Akslen LA. Loss of nuclear p16 protein expression is not associated with promoter methylation but defines a subgroup of aggressive endometrial carcinomas with poor prognosis. Clin Cancer Res. 2000;6(1):153-9. Epub 2000/02/03.
25. Nakashima R, Fujita M, Enomoto T, Haba T, Yoshino K, Wada H, et al. Alteration of p16 and p15 genes in human uterine tumours. British journal of cancer. 1999;80(3-4):458-67. Epub 1999/07/17.
26. Steinbakk A, Malpica A, Slewa A, Skaland I, Gudlaugsson E, Janssen EA, et al. Biomarkers and microsatellite instability analysis of curettings can predict the behavior of FIGO stage I endometrial endometrioid adenocarcinoma. Mod Pathol. 2011;24(9):1262-71. Epub 2011/05/10.
27. Alvarez T, Miller E, Duska L, Oliva E. Molecular profile of grade 3 endometrioid endometrial carcinoma: is it a type I or type II endometrial carcinoma? Am J Surg Pathol. 2012;36(5):753-61. Epub 2012/04/14.
28. Horree N, van Diest PJ, Sie-Go DM, Heintz AP. The invasive front in endometrial carcinoma: higher proliferation and associated derailment of cell cycle regulators. Hum Pathol. 2007;38(8):1232-8. Epub 2007/05/11.
29. Gemma A, Takenoshita S, Hagiwara K, Okamoto A, Spillare EA, McMemamin MG, et al. Molecular analysis of the cyclin-dependent kinase inhibitor genes p15INK4b/MTS2, p16INK4/MTS1, p18 and

p19 in human cancer cell lines. International journal of cancer Journal international du cancer. 1996;68(5):605-11. Epub 1996/11/27.

30. Herman JG, Merlo A, Mao L, Lapidus RG, Issa JP, Davidson NE, et al. Inactivation of the CDKN2/p16/MTS1 gene is frequently associated with aberrant DNA methylation in all common human cancers. Cancer Res. 1995;55(20):4525-30. Epub 1995/10/15.
31. Attardi LD, DePinho RA. Conquering the complexity of p53. Nature genetics. 2004;36(1):7-8. Epub 2004/01/01.
32. Saegusa M, Hashimura M, Suzuki E, Yoshida T, Kuwata T. Transcriptional up-regulation of Sox9 by NF-kappaB in endometrial carcinoma cells, modulating cell proliferation through alteration in the p14(ARF)/p53/p21(WAF1) pathway. The American journal of pathology. 2012;181(2):684-92. Epub 2012/06/16.
33. Horree N, van Diest PJ, van der Groep P, Sie-Go DM, Heintz AP. Progressive derailment of cell cycle regulators in endometrial carcinogenesis. J Clin Pathol. 2008;61(1):36-42. Epub 2007/05/08.
34. Jacks T, Weinberg RA. Cell-cycle control and its watchman. Nature. 1996;381(6584):643-4. Epub 1996/06/20.

CHAPTER 8

L1CAM EXPRESSION IN ENDOMETRIAL CARCINOMAS IS OF PROGNOSTIC VALUE AND SUPPORTS CORRECT HISTOPATHOLOGIC CLASSIFICATION

Yvette P. Geels[1], Johanna M.A. Pijnenborg[2], Bart B.M. Gordon[1], Mina Fogel[3], Peter Altevogt[4], Rina Masadah[5], Johan Bulten[6], Léon C. van Kempen[6,7], Leon F.A.G. Massuger[1]

[1] Department of Obstetrics and Gynecology, Radboud University Nijmegen, Medical Center, The Netherlands
[2] Department of Obstetrics and Gynecology, TweeSteden Hospital, Tilburg, The Netherlands
[3] Department of Pathology, Kaplan Medical Center, Rehovot, Israel
[4] Tumor Immunology Program, D015, German Cancer Research Center, D-69120 Heidelberg, Germany
[5] Department of Pathology, Hasanuddin University Hospital, Makassar, Indonesia
[6] Department of Pathology, Radboud University Nijmegen, Medical Center, The Netherlands
[7] Department of Pathology, McGill University / Jewish General Hospital, Montreal, QC, Canada

Submitted

Abstract

Objective: Endometrial carcinomas are classified in two subtypes. Type I tumors are endometrioid endometrial carcinomas (EECs), associated with estrogen stimulation and generally with good prognosis. Type II tumors represent non-endometrioid endometrial carcinomas (NEECs) with poor prognosis. However, 20% of the EECs are associated with poor clinical outcome despite a favorable histology. The aim of this study was to determine if L1CAM expression, a recently reported biomarker for aggressive tumor behavior in endometrial carcinoma, was associated with clinicopathologic features of EECs.

Methods: The immunohistochemical expression of L1CAM was determined in 103 EEC patients diagnosed from 1993-2009 in the Radboud University Medical Centre Nijmegen.

Results: Eighteen L1CAM positive EECs were identified. Surprisingly, review of the diagnostic slides revealed that 11 of the 18 L1CAM positive tumors contained a serous- or mixed carcinoma component that was previously not reported. L1CAM expression was associated with older age of the patient, poor tumor grade, and lymphovascular space invasion. A worse five year progression-free survival rate was observed for patients with L1CAM positive tumors (55.6% for the L1CAM positive group, compared to 83.3% for the L1CAM negative group P=0.01), but L1CAM expression was not an independent determinant of survival. Significant predictors of survival were high FIGO stage (III-IV) (HR 4.68, 95% CI 1.88-11.66), lymphovascular space invasion (HR 3.91, 95% CI 1.56-9.75), and NEEC (HR 4.12, 95% CI 1.38-12.31).

Conclusion: We conclude that L1CAM expression analysis supports correct classification of endometrial carcinomas and that it carries prognostic value for histologically confirmed EEC.

Introduction

Endometrial carcinoma is the most common gynecologic malignancy in industrialized nations.[1] Worldwide it is the third most common malignancy of the female genital tract with an incidence of 198,783, and a mortality rate of 50,327 annually.[2] The majority of endometrial carcinomas is classified as type I endometrioid endometrial carcinoma (EEC), and is related to unopposed estrogenic stimulation due to obesity, or exogenous hormone use. EEC originates from hyperplastic endometrium, and generally has a good prognosis. Type II carcinoma represents non-endometrioid endometrial carcinoma (NEEC), like uterine papillary serous carcinoma (UPSC) and clear cell carcinoma, and carries a high risk of disease progression.[3]

There are differences with respect to the molecular pathways that are important in the development of these different types of endometrial carcinomas. Type I carcinomas are characterized as diploid tumors, with the presence of estrogen-, and progesterone receptors, *PTEN* alterations, microsatellite instability (MSI), mutations of *KRAS*, and *CTNNB1*. Type II carcinomas on the contrary, are often aneuploid, and show over expression of p53 and Her2/neu.[4-6] Yet, about 20% of the individual cases does not fit within this dualistic model: EECs associated with poor clinical outcome and atrophic endometrium.[5,7,8] This group of endometrial carcinomas are either misclassified based on their histologic appearance, or are inherently different despite truly morphological and clinical characteristics of EEC.

Recently, expression of L1 cell adhesion molecule (L1CAM) has been associated with aggressive subtypes of endometrial carcinoma.[9,10] Moreover, L1CAM has shown to be of great importance for the prediction of clinical outcome of FIGO stage I, histologically confirmed EECs.[11] L1CAM is a neural cell recognition molecule, implicated in embryonic brain development.[12] It is a member of the immunoglobulin super family, with a structure of six immunoglobulin like domains, five fibronectin type III like domains, a transmembrane stratch, and a highly conserved cytoplasmic component.[13] L1CAM has an important role in the regulation of cell-cell interactions in neurohistogenesis, including axon outgrowth, neuronal migration, and regeneration after trauma.[12,14] In carcinoma cell lines, L1CAM over expression augments cell motility and tumor growth. In addition to endometrial carcinoma, L1CAM expression is associated with poor prognosis in melanoma, ovarian-, breast-, and colon carcinoma.[15-21]

The current study was conducted in order to identify the clinicopathologic features of L1CAM positive EECs, and to assess the prognostic value of L1CAM in EEC patients.

Materials & methods

Patients and tissue specimen

The nationwide network and registry of histology and cytopathology in the Netherlands (Pathologisch Anatomisch Landelijk Geautomatiseerd Archief: PALGA) was used to search for patients diagnosed, and surgically treated with hysterectomy and bilateral oophorectomy at the Radboud University Nijmegen Medical Centre (RUNMC) for EEC between January 1993 and January 2009. The terms “uterus” and “endometrioid carcinoma” were used to search through the PALGA database. Clinical data were collected by studying the medical charts. Age, menopausal state, body mass index (BMI), parity, use of estrogen, treatment, stage of disease, date of recurrence of disease, date of death, and the cause of death were registered. Stage of disease was based on the 1988 International Federation of Gynecology and Obstetrics (FIGO) staging system .[22] Four to eight representative slides of all patients were retrieved from the pathology archive and used for review. Review was done systematically including the following items: tumor grade, depth of myometrial invasion (MI), the presence of vascular space invasion either in lymphatic or blood vessels (LVSI), and the histologic type.[23] Review was performed independently by an experienced pathologist (RM) and an expert gyneco-pathologist (JB), who were unaware of the original pathology report, and the clinical outcome of the patient. Initial diagnosis was compared with the diagnosis after review. In case of discrepancy, the final diagnosis was obtained by consensus between the two pathologists. The EECs were also included in the study of Zeimet *et al*.[11] Immunohistochemical analysis of L1CAM was performed on sections of all endometrial carcinomas. The stained sections were analyzed by an independent pathologist who was not aware of the clinical outcome of the patients. Positive staining was defined as >10% immunoreactivity in any section derived from the tumor.

Antibodies and Immunohistochemistry

A monoclonal antibody to L1CAM (L1-40.10) was obtained after immunization of mice with human L1-Fc protein comprising the ectodomain of L1.[24] Staining was performed as

described previously.[11] Briefly, following EDTA antigen retrieval, sections were stained using the automated I6000 immunostainer (Biogenics, San Ramos, California, USA), staining of tissue was visualized using 3,3'-Diaminobenzidine (Zymed lab. California, USA) as substrate, and counterstained with Mayer's haematoxylin.

Statistical analysis

Differences in age, body mass index (BMI), FIGO stage, tumor histology, tumor grade, and LVSI between the group of patients with L1CAM positive and L1CAM negative tumors were tested for statistical significance using the Pearson's Chi-Square (χ^2) test or the Fisher's exact test, and Mann-Whitney test. Survival analyses were performed to study the progression free survival (PFS), and the disease specific survival (DSS). PFS was calculated from the date of surgery until the last date of progression free follow-up. DSS was calculated from the date of surgery until the date of death. Standard Kaplan-Meier curves were used to plot the survival estimates. Differences between curves were statistically tested using the log rank test. All *P*-values presented are two-sided, and associations were considered significant if the *P*-value was less than 0.05. The prognostic impact of the variables age, FIGO stage, tumor grade, MI, LVSI, histologic type, and L1CAM status were analyzed by using univariable and multivariable Cox proportional hazards models. The forward stepwise method was used for selection procedures for multivariable Cox proportional hazards models. These results were expressed as hazard ratio's (HR) with their 95% confidence intervals (95% CI). All statistical analyses were performed using the software package SPSS 18.0 for Microsoft Windows (SPSS Inc).

Ethical committee approval

The Research Ethics Committee of the Radboud University Nijmegen Medical Centre declared that the study protocol is in accordance with the applicable rules concerning the review of research ethics committees and informed consent.

Results

Patient characteristics and treatment

Between January 1993 and January 2009, 103 patients with EEC were retrieved, and included for analysis. Patient characteristics are presented in Table 1. The median age was 63 years, the majority of the patients was postmenopausal, and diagnosed at an early FIGO stage. Information for BMI calculation at diagnosis was available for 81 patients. The median BMI of these 81 patients was 28.9 kg/m^2. All patients underwent abdominal hysterectomy and bilateral salpingo-oophorectomy. Lymph node dissection was omitted in 80 cases that were without clinical suspicion of FIGO stage II or more, as recommended by the Dutch guidelines for endometrioid endometrial cancer treatment.[25] In only one patient lymph node metastases were identified. Forty patients received additional treatment; 39 patients received radiotherapy, one patient diagnosed as FIGO stage IV received adjuvant chemotherapy. Radiotherapy was either vaginal brachytherapy (N=11), external beam radiotherapy (N=21), or a combination of both (N=7). The five year DSS rate was 88.8%, the five year PFS rate was 77.7%. The median time of follow up was 57 months (range 0-148).

Review of the histologic slides

After review, the initial diagnosis was adjusted in 31 patients. In 25 cases tumor grade changed: upgrading from grade 1 to 2 (N=8), from grade 2 to 3 (N=10), from grade 1 to 3 (N=1), downgrading from grade 3 to 2 (N=3), and 2 to 1 (N=3). In 11 cases the histology was classified different than the initial diagnosis of EEC. Five tumors were finally diagnosed as uterine papillary serous carcinoma (UPSC), three tumors were diagnosed as mixed carcinoma, and three as undifferentiated carcinomas. The diagnosis of mixed carcinoma was defined when at least 10% of a second component was present. The differences in initial diagnosis, and diagnosis after review of patients with changed histology are shown in Table 2.

Immunohistochemistry

In the total study population of 103 carcinomas, 18 patients showed L1CAM positive staining in the tumor. All 11 NEECs, and seven confirmed EECs were L1CAM positive. L1CAM staining showed variable intensity. Staining had a tendency to more intensity at the invasive

Table 1: Clinical and pathologic characteristics (after review) in the total population (N=103).

Clinico-pathologic characteristics	Total (N=103) Median/N (range/%)
Median age (years)	63 (24-86)
Postmenopausal	
No	22 (21.3)
Yes	73 (70.9)
Unknown	8 (7.8)
Median BMI* (kg/m^2)	28.9 (18.7-53.6)
Lymph nodes	
Positive	1 (1.0)
Negative	22 (21.3)
Unknown	80 (77.7)
Adjuvant radiotherapy	
Yes	39 (37.9)
No	64 (62.1)
Adjuvant chemotherapy	
Yes	1 (1.0)
No	102 (99.0)
FIGO stage**	
Low (I-II)	84 (81.6)
High (III-IV)	19 (18.4)
Tumor grade	
Low (1-2)	78 (75.7)
High (3)	25 (24.3)
Myometrial Invasion	
<50%	61 (59.2)
>50%	42 (40.8)
Lymphovascular Space Invasion	
Not present	80 (77.7)
Present	23 (22.3)
Histology	
Endometrioid	92 (82.3)
Non- endometrioid	11 (10.7)
Adjuvant radiotherapy	
No	64 (62.1)
Yes	39 (37.9)
Adjuvant chemotherapy	
No	100 (99.0)
Yes	1 (1.0)
Five year disease specific survival rate	88.8%
Five year progression free survival rate	77.7%
Median follow up (months)	57 (0-148)

*Body Mass Index **1988 International Federation of Gynecology and Obstetrics staging system

Table 2: Initial diagnosis and diagnosis after review of cases with a changed diagnosis of EEC into NEEC.

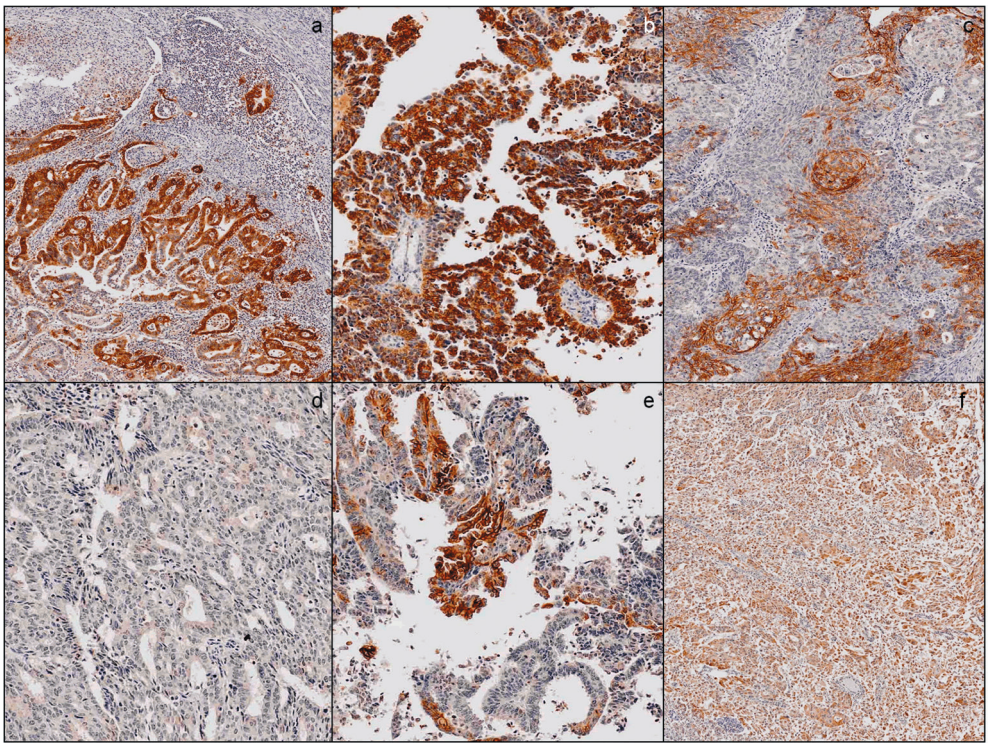
	Before revision		After revision	
Case	Histology	Grade	Histology	Grade
6	Endometrioid	3	Serous	3
12	Endometrioid	3	Serous	3
14	Endometrioid	2	Undifferentiated	3
18	Endometrioid	2	Serous	3
21	Endometrioid	2	50% endometrioid / 50% serous	3
32	Endometrioid	3	Serous	3
45	Endometrioid	2	50% endometrioid / 50% serous	3
47	Endometrioid	3	Undifferentiated	3
48	Endometrioid	3	Serous	3
50	Endometrioid	2	50% serous/ 50% clear cell	3
53	Endometrioid	3	Undifferentiated	3

front (Figure 1A). The five tumors, diagnosed as pure UPSC after review of the histological slides, showed positive L1CAM staining throughout a major part of the tumor specimens. A representative example is shown in Figure 1B. Figure 1C shows an example of L1CAM positive staining in EEC. In the two mixed carcinomas with 50% serous component, and 50% endometrioid component, the serous component was strongly positive, while the endometrioid component was weakly positive, or L1CAM negative (Figure 1D and 1E). Figure 1F shows L1CAM positive staining in an undifferentiated carcinoma, which is diffuse positive trough the tumour specimen.

L1CAM expression and clinicopathologic characteristics

The clinical and pathological characteristics of L1CAM negative and L1CAM positive patients after review are summarized in Table 3. Adjuvant treatment and mean time of follow up were equal in both groups. Patients with L1CAM negative tumors were significantly younger compared to patients with L1CAM positive tumors. There was no significant difference between L1CAM negative and L1CAM positive tumors with respect to menopausal state, BMI, and FIGO stage.

Figure 1: L1CAM expression in endometrial carcinoma.



L1CAM expression at the invasive front of endometrioid endometrial carcinoma (A), papillary serous carcinoma (B), and endometrioid endometrial carcinoma with L1CAM positive staining (C). A mixed carcinoma (50% endometrioid : 50% serous) with no L1CAM expression in the endometrioid component (D) but with L1CAM expression in the serous component (E). L1CAM expression in an undifferentiated carcinoma (F).

In addition, comparison of L1CAM negative and positive tumors revealed significant differences in distribution of tumor grade and frequency of lymphovascular invasion. No significant difference was found concerning myometrial invasion in both groups. The five year PFS rate was 82.3% in patients with EEC compared to 44.4% in patients with NEEC (Figure 2A, $P<0.01$). The five year DSS rate was 89.8%, and 50.0% respectively (Figure 2B, $P<0.01$). Comparing survival in L1CAM negative and L1CAM positive patients resulted in a five year PFS rate of 83.3% and 55.6% respectively (Figure 2C, $P<0.01$), and a five year DSS rate of 91.3% and 76.0% respectively (Figure 2D, $P=0.04$).

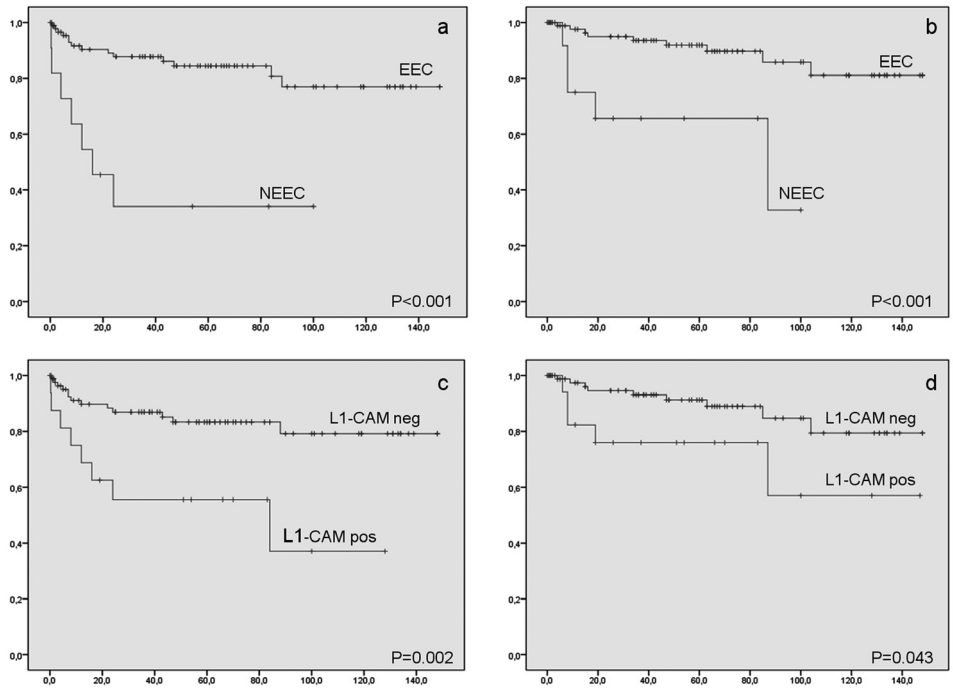
Importantly, seven of 92 histopathologically confirmed EECs (7.6%) showed L1CAM expression. Comparing patients with L1CAM negative status and L1CAM positive status in

Table 3: Clinical and pathologic characteristics (after review) in the total population, the L1CAM negative and the L1CAM positive tumors.

Clinico-pathologic characteristics	L1CAM negative	L1CAM positive	*P*-value
	N=85 (range/%)	N=85 (range/ %)	
Mean age (years)	59.7 (24-86)	68.2 (47-81)	<0.01
Postmenopausal			
No	21 (26.3)	1 (6.7)	
Yes	59 (73.7)	14 (93.3)	0.18
Median BMI in kg/m^2 (range)	29.3 (18.7-53.6)	27.1 (19.8-47.1)	0.12
Lymph nodes			
Positive	1 (5.6)	0 (27.8)	
Negative	17 (94.4)	5 (100.0)	1.00
FIGO stage*			
Low (I-II)	71 (83.5)	13 (72.2)	
High (III-IV)	14 (16.5)	5 (27.8)	0.32
Tumor grade			
Low (1-2)	75 (88.2)	3 (16.7)	
High (3)	10 (11.8)	15 (83.3)	<0.01
Myometrial Invasion			
<50%	54 (63.5)	7 (38.9)	
>50%	31 (36.5)	11 (61.1)	0.06
Lymphovascular Space Invasion			
Not present	73 (85.9)	7 (38.9)	
Present	12 (14.1)	11 (61.1)	<0.01
Histology			
Endometrioid	85 (100)	7 (38.9)	
Non- endometrioid	0 (0.0)	11 (61.1)	<0.01
Radiotherapy			
No	49 (57.6)	13 (81.3)	
Yes	36 (42.4)	3 (18.8)	0.10
Mean follow-up (months)	60.4 (0.4-148.0)	51.1 (0-147.0)	0.40

*1988 International Federation of Gynecology and Obstetrics staging system

Figure 2: Survival characteristics of endometrial carcinoma patients.



Progression free survival (A) and disease specific survival (B) of EEC patients compared to NEEC patients in months. Progression free survival (C) and disease specific survival (D) of patients with L1CAM positive or negative tumors in months.

the group with only EEC patients did not result in significant differences in survival due to this limited number of L1CAM positive EEC cases (data not shown).

The results of the univariable- and multivariable analyses of the risk of recurrence or progression of disease and death as a consequence of disease are shown in Table 4 and 5. High FIGO stage (FIGO III-IV), poor tumor grade (grade 3), the presence of lymphovascular space invasion, NEEC, and L1CAM positivity were significant predictors of PFS. Significant predictors of DSS were high FIGO stage (III-IV), tumor grade (grade 3), myometrial invasion >50%, the presence of lymphovascular space invasion, NEEC, and L1CAM positive staining. When entering these significant factors in a multivariable model, only high FIGO stage (III-IV), the presence of LVSI, and NEEC remained significant predictors of both poor PFS and DSS. Furthermore, there was a strong correlation between L1CAM status and histological type after multivariable analyses of prognostic variables related with PFS and DSS.

Table 4: Hazard Ratio's (HR) with 95% Confidence Interval (95% CI) of the prognostic variables for recurrence and death as a consequence of disease after univariable Cox proportional hazards models .

Prognostic variable		Recurrence		Death of disease
	N	HR (95% CI)	N	HR (95% CI)
Age				
< 60	48	1.00 (reference)	47	1.00 (reference)
> 60	54	1.41 (0.59- 3.37)	51	1.57 (0.54- 4.54)
FIGO stage*				
low: I-II	83	1.00 (reference)	81	1.00 (reference)
high: III-IV	19	**4.02 (1.66- 9.75)**	17	**3.99 (1.33- 12.00)**
Tumor grade				
Low: 1-2	78	1.00 (reference)	75	1.00 (reference)
High: 3	24	**3.80 (1.61- 9.00)**	23	**4.04 (1.39- 11.74)**
Myometrial Invasion				
<50%	60	1.00 (reference)	60	1.00 (reference)
>50%	42	2.28 (0.96- 5.42)	38	**3.42 (1.14- 10.25)**
Lymphovascular space Invasion				
Not present	79	1.00 (reference)	76	1.00 (reference)
Present	23	**4.13 (1.73- 9.86)**	22	**4.20 (1.45-12.11)**
Histology				
Endometrioid	92	1.00 (reference)	89	1.00 (reference)
Non-endometrioid	10	**4.67 (1.70- 12.81)**	9	**6.86 (2.09- 22.54)**
L1CAM				
Negative	85	1.00 (reference)	82	1.00 (reference)
Positive	17	**3.69 (1.53- 8.93)**	16	**3.09 (1.03- 9.23)**

* 1988 International Federation of Gynecology and Obstetrics staging system

Table 5: Adjusted Hazard Ratio's (HR) of the prognostic variables for recurrence and death as a consequence of disease after multivariable Cox proportional hazards models with forward selection procedures.

Prognostic variable		Recurrence		Death of disease
	N	HR (95% CI)	N	HR (95% CI)
FIGO stage*				
Low: I-II	83	1.00 (reference)	81	1.00 (reference)
High: III-IV	19	4.68 (1.88-11.66)	17	4.17 (1.30-13.39)
Tumor grade				
Low: 1-2		NS**		NS
High: 3				
Myometrial Invasion				
< 50%		NS		NS
> 50%				
Lymphovascular Space Invasion				
Not present	79	1.00 (reference)	76	1.00 (reference)
Present	23	3.91 (1.56- 9.75)	22	3.79 (1.20-12.00)
Histology				
Endometrioid	92	1.00 (reference)	89	1.00 (reference)
Non-endometrioid	10	4.12 (1.38-12.31)	9	5.15 (1.34-19.78)
L1CAM				
Negative		NS		NS
Positive				

* 1988 International Federation of Gynecology and Obstetrics staging system **Not Selected

Discussion

This study was conducted to determine whether L1CAM expression in EECs is related to pathologic features and clinical outcome. Our study revealed L1CAM expression in 18/103 (17%) of the tumors originally diagnosed as EEC. Eleven of these cases were reclassified after expert review of the original diagnostic slides. Seven L1CAM positive EECs were identified.

The study group of 103 endometrial carcinoma patients is representative for the population diagnosed with endometrial cancer, with a median age at diagnosis of 63 years, a median BMI of 28.9 kg/m^2, a majority of early stage disease, and a minority of poorly differentiated tumors.[3,26] However, the number of patients with EEC during the selected period of time was less than expected. This is probably due to the fact that the search terms “uterus” and “endometrioid carcinoma” in the PALGA database do not cover all EEC patients. Since selection of patients was accomplished using a search question we believe the studied group can be considered as a representative sampling.

Furthermore, due to the retrospective character of the study over a long period of time, and the fact that UPSC has been recognized since the turn of the century, it is likely that UPSC has been misdiagnosed in the period till 2001.[3] This is supported by our findings that all missed diagnosis of UPSC were diagnosed between 1993 and 2001. Improved awareness of the pathologist of the existence of UPSC nowadays, decreases the number of UPSCs mistaken for EECs.

A recent multicentre study of L1CAM expression in 1021 histologically confirmed EECs demonstrated that L1CAM expression in EEC is an independent predictor of clinical outcome. A small percentage of these cases showed areas of non-endometrioid differentiation in less than 10% of the tumor, and this was associated with L1CAM positive staining.[11] The molecular mechanism that drives L1CAM expression and how this contributes to poor prognosis remains largely elusive.

Several studies have shown that L1CAM expression is associated with aggressive carcinoma subtypes and tumor progression.[10,15-21] In serous ovarian and endometrial carcinomas, L1CAM expression is frequently present.[10] If L1CAM expression is present in EECs, it is associated with poor tumor differentiation, absence of estrogen and progesterone receptors, and loss of E-cadherin expression.[9] In our study, L1CAM expression was a predictor of poor progression free survival in univariable analysis. In multivariable analysis L1CAM was not

a significant predictor of survival, but was strongly correlated with NEEC, which was an independent predictor of poor clinical outcome.

Consensus on the treatment of endometrial cancer patients is lacking and molecular markers for risk stratification are needed. Ideally, patients who would benefit from a more aggressive treatment strategy such as lymphadenectomy, should be identified pre-operatively. Therefore, prognostic immunohistochemical markers should be used on endometrial specimens obtained with dilatation and curettage or pipelle. A set of markers including survivin, p21, and p53 has been suggested to predict prognosis in early stage endometrial carcinoma.[27] Moreover, p53 and bcl-2 expression on pre-operative biopsies of the endometrium were found to be predictive for lymph node metastases.[28] In this pre-operative setting L1CAM could be a useful additional tool. It not only helps to identify NEECs, but it also identifies those EEC patients who are at high risk of disease progression. The prognostic value of a multiplexed biomarker analysis needs to be determined for risk stratification of endometrial cancer patients.

Similarly, the predictive value of L1CAM expression in the adjuvant setting needs to be determined. The use of adjuvant chemotherapy for the treatment of endometrial carcinoma is subject of debate. In two randomized trials no difference was found between adjuvant chemotherapy and adjuvant radiotherapy in high-risk patients.[29,30] Combination of chemotherapy and radiotherapy is reported to improve progression free survival compared to radiotherapy only, yet, no differences were found in overall survival.[31] Adjuvant chemotherapy is advised in NEEC patients with myometrial invasion and/or lymph node involvement.[32] Our findings that L1CAM positive ECC is associated with poor PFS and DSS warrants further investigation into the predictive value of L1CAM expression for adjuvant chemotherapy for EEC patients.

Determination of histology in poorly differentiated tumors can be challenging. Yet, accurate diagnosis of endometrial carcinomas is of great clinical importance, given the prognostic and therapeutic implications.[33,34] In our study group of 103 EECs, 11 patients were diagnosed incorrectly, and were found to be NEEC patients. None of these 11 patients were properly staged, or received adjuvant chemotherapy. Three patients were diagnosed with FIGO stage IV and were never free of disease after primary treatment. Three other patients had distant recurrence of disease within two years. These six patients could have benefit from adjuvant

chemotherapy. Equally for radiotherapy, an incorrect histologic diagnosis resulted in under treatment in three cases.

Taken this together, it is of great importance that in poorly differentiated tumors differentiation between NEEC and EEC is appropriate. In this study, the mixed endometrial carcinomas showed positive L1CAM staining in the serous component, whereas the endometrioid component was L1CAM negative. These observations have been described previously.[9] In literature, several immunohistochemical markers are used to support the diagnosis of UPSC, i.e. over expression of p53, and p16, as well as loss of hormone receptors. Additionally, loss of PTEN expression supports the diagnosis of tumor grade 3 EEC.[35,36] L1CAM might be a useful marker to add to a panel of markers to distinguish EEC from NEEC. In addition, L1CAM staining was observed to be strongest at the invasive front, which confirms the suggestion that L1CAM is important for tumor invasion. However in the to date largest reported study on L1CAM expression in EEC, this particular pattern of L1CAM staining was not observed.[11]

In conclusion, L1CAM is significantly associated with non-endometrioid histology and other clinicopathologic factors predicting poor survival. This makes L1CAM a potential marker for pre-operative identification of patients needing aggressive surgical and/or adjuvant treatment. Furthermore, distinction between NEEC and poorly differentiated EEC is challenging, but of great clinical importance. L1CAM could be a useful marker in the detection of non-endometrioid histology and of EEC with poor prognosis. A large prospective study is required to determine the clinical implications of L1CAM in endometrial carcinomas.

References

1. Global Cancer Facts & Figures. 2008.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005;55(2):74-108. Epub 2005/03/12.
3. Ellenson HL, Ronnett BM, Soslow RA, Zaino RJ, Kurman RJ. Endometrial carcinoma. In: Kurman RJ, Ronnett BM, Ellenson HL, editors. Blaustein's Pathology of the Female Genital Tract. Sixth ed. New York: Springer; 2011. p. 393-452.
4. Prat J, Gallardo A, Cuatrecasas M, Catasus L. Endometrial carcinoma: pathology and genetics. Pathology. 2007;39(1):72-87. Epub 2007/03/17.
5. Engelsen IB, Akslen LA, Salvesen HB. Biologic markers in endometrial cancer treatment. APMIS. 2009;117(10):693-707. Epub 2009/09/25.
6. Markova I, Pilka R, Duskova M, Zapletalova J, Kudela M. [Prognostic significance of clinic pathological and selected immunohistochemical factors in endometrial cancer]. Ceska Gynekol. 2010;75(3):193-9. Epub 2010/08/25. Prognosticky vyznam klinickopatologickych a vybranych imunohistochemickych faktoru u karcinomu endometria.
7. Lax SF. Molecular genetic changes in epithelial, stromal and mixed neoplasms of the endometrium. Pathology. 2007;39(1):46-54. Epub 2007/03/17.
8. Geels YP, Pijnenborg JM, van den Berg-van Erp SH, Bulten J, Visscher DW, Dowdy SC, et al. Endometrioid endometrial carcinoma with atrophic endometrium and poor prognosis. Obstet Gynecol. 2012;120(5):1124-31. Epub 2012/10/24.
9. Huszar M, Pfeifer M, Schirmer U, Kiefel H, Konecny GE, Ben-Arie A, et al. Up-regulation of L1CAM is linked to loss of hormone receptors and E-cadherin in aggressive subtypes of endometrial carcinomas. J Pathol. 2010;220(5):551-61. Epub 2010/01/16.
10. Fogel M, Gutwein P, Mechtersheimer S, Riedle S, Stoeck A, Smirnov A, et al. L1 expression as a predictor of progression and survival in patients with uterine and ovarian carcinomas. Lancet. 2003;362(9387):869-75. Epub 2003/09/19.
11. Zeimet AG, Reimer D, Huszar M, Winterhoff B, Puistola U, Abdel Azim S, et al. L1CAM in Early-Stage Type I Endometrial Cancer: Results of a Large Multicenter Evaluation. J Natl Cancer Inst. 2013. Epub 2013/06/20.
12. Brummendorf T, Kenwrick S, Rathjen FG. Neural cell recognition molecule L1: from cell biology to human hereditary brain malformations. Curr Opin Neurobiol. 1998;8(1):87-97. Epub 1998/05/06.
13. Moos M, Tacke R, Scherer H, Teplow D, Fruh K, Schachner M. Neural adhesion molecule L1 as a member of the immunoglobulin superfamily with binding domains similar to fibronectin. Nature. 1988;334(6184):701-3. Epub 1988/08/25.
14. Maness PF, Schachner M. Neural recognition molecules of the immunoglobulin superfamily: signaling transducers of axon guidance and neuronal migration. Nat Neurosci. 2007;10(1):19-26. Epub 2006/12/27.
15. Gavert N, Conacci-Sorrell M, Gast D, Schneider A, Altevogt P, Brabletz T, et al. L1, a novel target of beta-catenin signaling, transforms cells and is expressed at the invasive front of colon cancers. J Cell Biol. 2005;168(4):633-42. Epub 2005/02/18.

16. Boo YJ, Park JM, Kim J, Chae YS, Min BW, Um JW, et al. L1 expression as a marker for poor prognosis, tumor progression, and short survival in patients with colorectal cancer. Ann Surg Oncol. 2007;14(5):1703-11. Epub 2007/01/11.
17. Gast D, Riedle S, Issa Y, Pfeifer M, Beckhove P, Sanderson MP, et al. The cytoplasmic part of L1-CAM controls growth and gene expression in human tumors that is reversed by therapeutic antibodies. Oncogene. 2008;27(9):1281-9. Epub 2007/10/24.
18. Fogel M, Huszar M, Altevogt P, Ben-Arie A. L1 (CD171) as a novel biomarker for ovarian and endometrial carcinomas. Expert Rev Mol Diagn. 2004;4(4):455-62. Epub 2004/07/01.
19. Fogel M, Mechtersheimer S, Huszar M, Smirnov A, Abu-Dahi A, Tilgen W, et al. L1 adhesion molecule (CD 171) in development and progression of human malignant melanoma. Cancer Lett. 2003;189(2):237-47. Epub 2002/12/20.
20. Altevogt P, Fogel M. The role of L1 in the progression of ovarian carcinomas. Zentralbl Gynakol. 2004;126(5):323-5. Epub 2004/10/13.
21. Schroder C, Schumacher U, Fogel M, Feuerhake F, Muller V, Wirtz RM, et al. Expression and prognostic value of L1-CAM in breast cancer. Oncol Rep. 2009;22(5):1109-17. Epub 2009/09/30.
22. Creasman WT. New gynecologic cancer staging. Obstet Gynecol. 1990;75(2):287-8. Epub 1990/02/01.
23. Silverberg SG, Mutter GL, Kurman RJ, Kubik-Huch RA, Nogales F, Tavassoli FA. Tumors of the uterine corpus: epithelial tumors and related lesions. In: Tavassoli FA, Stratton FR, editors. WHO classification of tumors: pathology and genetics of tumors of the breast and female genital organs: World Health Organisation; 2003. p. 221-32.
24. Oleszewski M, Beer S, Katich S, Geiger C, Zeller Y, Rauch U, et al. Integrin and neurocan binding to L1 involves distinct Ig domains. J Biol Chem. 1999;274(35):24602-10. Epub 1999/08/24.
25. www.oncoline.nl. 2011.
26. Crum CP, Duska LR, Lee KR, Mutter GL. Adenocarcinoma, carcinosarcoma, and other epithelial tumors of the endometrium. Diagnostic Gynecologic and Obstetric Pathology: Elsevier Saunders; 2004. p. 546-602.
27. Steinbakk A, Skaland I, Gudlaugsson E, Janssen EA, Kjellevold KH, Klos J, et al. The prognostic value of molecular biomarkers in tissue removed by curettage from FIGO stage 1 and 2 endometrioid type endometrial cancer. Am J Obstet Gynecol. 2009;200(1):78 e1-8. Epub 2008/11/04.
28. Mariani A, Sebo TJ, Katzmann JA, Roche PC, Keeney GL, Lesnick TG, et al. Endometrial cancer: can nodal status be predicted with curettage? Gynecol Oncol. 2005;96(3):594-600. Epub 2005/02/22.
29. Maggi R, Lissoni A, Spina F, Melpignano M, Zola P, Favalli G, et al. Adjuvant chemotherapy vs radiotherapy in high-risk endometrial carcinoma: results of a randomised trial. British journal of cancer. 2006;95(3):266-71. Epub 2006/07/27.
30. Susumu N, Sagae S, Udagawa Y, Niwa K, Kuramoto H, Satoh S, et al. Randomized phase III trial of pelvic radiotherapy versus cisplatin-based combined chemotherapy in patients with intermediate- and high-risk endometrial cancer: a Japanese Gynecologic Oncology Group study. Gynecol Oncol. 2008;108(1):226-33. Epub 2007/11/13.

31. Hogberg T, Signorelli M, de Oliveira CF, Fossati R, Lissoni AA, Sorbe B, et al. Sequential adjuvant chemotherapy and radiotherapy in endometrial cancer--results from two randomised studies. European journal of cancer (Oxford, England : 1990). 2010;46(13):2422 31. Epub 2010/07/14.
32. Boruta DM, 2nd, Gehrig PA, Fader AN, Olawaiye AB. Management of women with uterine papillary serous cancer: a Society of Gynecologic Oncology (SGO) review. Gynecol Oncol. 2009;115(1):142-53. Epub 2009/07/14.
33. Altrabulsi B, Malpica A, Deavers MT, Bodurka DC, Broaddus R, Silva EG. Undifferentiated carcinoma of the endometrium. Am J Surg Pathol. 2005;29(10):1316-21. Epub 2005/09/15.
34. Boruta DM, 2nd, Gehrig PA, Groben PA, Bae-Jump V, Boggess JF, Fowler WC, Jr., et al. Uterine serous and grade 3 endometrioid carcinomas: is there a survival difference? Cancer. 2004;101(10):2214-21. Epub 2004/09/29.
35. Alkushi A, Kobel M, Kalloger SE, Gilks CB. High-grade endometrial carcinoma: serous and grade 3 endometrioid carcinomas have different immunophenotypes and outcomes. Int J Gynecol Pathol. 2010;29(4):343-50. Epub 2010/06/23.
36. Darvishian F, Hummer AJ, Thaler HT, Bhargava R, Linkov I, Asher M, et al. Serous endometrial cancers that mimic endometrioid adenocarcinomas: a clinicopathologic and immunohistochemical study of a group of problematic cases. Am J Surg Pathol. 2004;28(12):1568-78. Epub 2004/12/04.

CHAPTER 9

GENERAL DISCUSSION

General Discussion

This thesis describes the histopathologic appearance of the endometrium of asymptomatic women and endometrioid endometrial carcinoma (EEC) patients in detail, and discusses the pathogenesis of EEC. Furthermore, it describes the role of several pathologic, and immunohistochemical (IHC) markers in the prediction of the clinical course of endometrial carcinoma patients. The relevance and possible clinical consequences of the findings of this thesis are discussed in this chapter.

The endometrium in postmenopausal women

It is expected that, in its natural course, the endometrium of premenopausal women shows a cyclic pattern, whereas in postmenopausal women the endometrium is atrophic.[1] In order to investigate endometrial carcinogenesis, the physiological conditions of the endometrium should be clear. In Chapter 2, it is shown that the endometrium of postmenopausal, asymptomatic women demonstrates focal proliferation or hyperplasia in 16 out of 48 patients (33%). Weak proliferation in the endometrium of asymptomatic postmenopausal women has been reported before.[2] Whether this proliferation is pathologic, and of clinical importance has yet to be determined. It is assumed that the transition of proliferative endometrium into atrophic endometrium after menopause is a gradual process,[1] marked by varying serum estradiol levels, decreased serum progesterone levels, and a potentially disturbed ovarian-pituitary-hypothalamic feedback relationship.[3,4] This gradual process could explain why years after menopause features of proliferative endometrium can still be found in the remaining inactive endometrium.

The focal presence of proliferative endometrium might indicate that the endometrium is only partially sensitive for the influence of circulating estrogens and progesterone. Possibly, within the same patient, the presence of Estrogen Receptors (ER) and Progesterone Receptors (PR) is heterogeneous in the endometrial surface. Yet, Sivridis *et al* performed immunohistochemical staining of postmenopausal endometrium and found positive ER and PR staining independent of whether the endometrium was active or inactive. However, they did find more evident staining patterns of epidermal growth factor receptor, proliferation by MIB1, and increased angiogenic activity in the stroma of weakly proliferative endometrium

when compared to inactive endometrium.[5] Future research of asymptomatic endometrium should give more insight into the mechanism of focal proliferation.

Carcinogenesis of endometrioid endometrial carcinoma

It is generally believed that endometrioid endometrial carcinomas are a consequence of unopposed estrogen stimulation. Their carcinogenesis follows a stepwise process, gradually originating from hyperplastic endometrium.[6-8] In Chapter 5, it is demonstrated that in 17% of patients with a grade 1 EEC tumor no, or only focal, hyperplasia was found in the background endometrium, indicating that this carcinogenic model does not explain fully the emergence of all EECs. However, it may be that these EECs in atrophic background endometrium developed in focal proliferative or hyperplastic areas. This hypothesis is supported by the finding of focal proliferation in further atrophic endometrium in Chapter 2. In addition, the expression pattern of IHC markers and mutations in *BRAF* and *PIK3CA* were comparable between EEC patients with hyperplastic and with atrophic endometrium. These findings suggest that part of the carcinogenesis is directed through the same pathways.

Nevertheless, in Chapter 6, *KRAS* mutation was found in only 2% of patients with atrophic background endometrium, and in 37% of patients with hyperplastic background endometrium. *KRAS* mutations are known to be an early event in endometrioid endometrial carcinogenesis and are found in a large percentage of patients with hyperplastic endometrium.[9,10] This means that the EECs with atrophic background endometrium might lack a crucial step in the current model of EEC carcinogenesis. In addition, *KRAS* mutation results in an upregulation of E-cadherin expression, and repressors of E-cadherin are found in endometrial carcinoma cell lines without *KRAS* mutation.[11,12] We found loss of E-cadherin expression in the carcinomas with atrophic background endometrium when compared to the carcinomas with background endometrial hyperplasia. Loss of E-cadherin expression, which is the most important cell-cell adhesion molecule in epithelial cells, is known to be a predictor of poor clinical outcome.[13-15] The carcinomas with atrophic endometrium showed a worse prognosis compared to the carcinomas with hyperplastic endometrium, which has been reported by other authors before.[16] These findings support the hypothesis that EECs with atrophic background endometrium partly develop through different pathways than

EECs with hyperplastic background endometrium. Possibly, this may be the so-called third type endometrial carcinoma.[16,17]

Prognostic value of pathologic markers

As pointed out in the introduction, prediction of the clinical course is extremely important in order to prevent under and overtreatment. Whereas prediction will be more and more supported by immunohistochemistry and molecular analyses, currently, most predictive models of endometrial carcinoma are still based on clinical and pathologic features,[18] of which some are analyzed in this thesis.

For the pre-operative setting, in Chapter 3 it is shown that the vast majority of patients with uterine papillary serous carcinoma have malignant endometrial cells in the cervical cytology, in contrast to only one third of the patients with endometrioid carcinomas. These results confirm the findings of other studies.[19,20] Cervical cytology could be a predictive tool for patients with endometrial carcinoma, since it may give a warning sign of a non-endometrioid type tumor. Furthermore, like in our study, the presence of malignant or atypical endometrial cells in cervical cytology has been associated with other predictors of poor survival such as stromal invasion of the cervix and advanced FIGO stage.[21] It has been demonstrated recently that mutations found in the hysterectomy specimens of endometrial carcinoma patients were also found in the liquid based Papanicolaou smears of the same patients.[22] Whether DNA analyses of PAP smears may be of clinical use in the detection of endometrial carcinoma or in the prediction of clinical behavior needs to be sorted out in future research.

For the post-operative setting, in Chapter 5 it is shown that atrophic background endometrium is an important predictor of clinical outcome. In current clinical practice, one or two slides of the endometrium adjacent to the carcinoma are evaluated. Unfortunately, it is not incorporated in the guidelines of pathologists to mention the nature of the background endometrium. Given the results of Chapter 6, the nature of the background endometrium should be mentioned in every pathology report.

Additionally, in the post-operative setting, myometrial invasion is known to be one of the most important predictors of recurrence of disease.[18,23,24] Therefore, to our opinion this parameter should be as reliable as possible. Currently, in the FIGO staging system,

myometrial invasion is described as more or less than 50% of the total myometrial thickness. Chapter 4 demonstrates that measuring the absolute depth of myometrial invasion and using a cut-off point of 4 mm predicts recurrence of disease and disease-free survival better than the currently used cut-off point of 50% of the total myometrial thickness. In literature, conflicting results about the best way of measuring myometrial invasion are reported. Some authors found absolute depth of myometrial invasion to be the best predictor,[25] others found the tumor-free distance to be a better prognostic indicator.[26-28] Nevertheless, we agree with these authors that an absolute cut-off value for myometrial invasion gives better predictive results than a percentage as cut-off value. Future research should sort out which way of measuring myometrial invasion is the best predictor of recurrence and which cut-off value should be used.

Prognostic value of IHC markers

In order to identify immunohistochemical markers for the prediction of clinical behavior of endometrial carcinomas, we attempted to gain more insight into the metastatic process of EECs in Chapter 7. Vandenput *et al* recently revealed changes in expression of PR, p53, and p-mTOR in the recurrent tumor when compared to the primary tumor. These results show that endometrial carcinoma biology changes over time.[29] We compared primary tumors with and without metastases, and primary tumors with the metastatic site to reveal how tumor biology changes in the metastatic process.

A loss of ER expression was found in the primary carcinoma of EEC patients with proven metastases (locoregional and distant) when compared to EEC patients without metastases. In addition, within the metastatic group, loss of ER and PR expression and gain of MIB1 expression was found in the primary tumor of patients with distant metastases in comparison with the primary tumor of patients with locoregional metastases. These findings emphasize again that hormone receptors play an important role in endometrioid carcinogenesis and are important predictors of survival. It also indicates that expression of cell-cycle regulator MIB1 seems to be an important step in the metastatic process for distant metastases.[30-32] The predictive use of ER, PR, and MIB1 is the most interesting for the clinician, as they contribute to the identification of carcinomas that have the potential to metastasize.

Gain of p16 and p21 expression was found in the metastatic site when compared to the primary tumor. *P16* is a tumor suppressor gene and part of the Rb (retinoblastoma associated protein pathway), which is an important suppressor of proliferation.[33] Changes in p16 expression were associated with aggressive endometrial carcinomas before. However, whether increased or decreased p16 expression is a predictor of poor survival is controversial.[34-36] In addition, the inactivation of *P21* leads to tumor progression, as it is a critical downstream effector in the *P53* specific pathway.[30,37,38] *P53* is one of the most prominent suppressors of proliferation.[39] Also for p21 results of expression patterns are conflicting in literature as both gain and loss of protein expression are associated with aggressive tumor behavior.[40-43] Nevertheless, taking these studies and our results together, alterations in *P16* and *P21* seem to increase the metastatic potential of endometrial carcinoma. Furthermore, changes in the pathways of proliferation suppressors p53 and Rb seem to be important steps in the metastatic process of EECs. The conflicting results of p16 and p21 expression need to be sorted out in future research in order to determine the clinical use of the IHC expression of these two proteins.

Chapter 8 focuses on a promising marker in the identification of aggressive endometrial carcinomas: L1CAM.[44] L1CAM was recently identified to be an independent predictor of survival in EECs specifically.[45] In this large multicentre cohort of over one thousand EEC patients, carcinomas with L1CAM expression showed a striking decrease in the disease free five-year survival rate when compared to L1CAM negative EECs. However, only 18% of this large cohort was L1CAM positive. As shown in Chapter 5, L1CAM was strongly associated with non-endometrioid histology in our series. It was previously shown that the percentage of L1CAM positive tumors was 75% in serous carcinomas and 16% in endometrioid carcinomas.[46] Taking this together, a L1CAM positive endometrial carcinoma may be either a carcinoma with non-endometrioid histology, or an endometrioid carcinoma with aggressive behavior. This makes L1CAM a useful marker for the prediction of clinical behavior of endometrial carcinomas.

The association of L1CAM with EECs with a poor clinical course led to the presumption that more L1CAM expression could be found in EECs with atrophic background endometrium when compared to the EECs with hyperplastic background endometrium. We did not find this difference in Chapter 6, but we did find a significant gain of expression in the serous carcinomas in Chapter 7. The low prevalence of L1CAM expression in EECs is a possible

explanation for this. In Chapter 8, of the seven L1CAM positive EECs, only one carcinoma had atrophic background endometrium. Of the other six carcinomas one had premenopausal proliferative, four had hyperplastic, and one had no background endometrium. It would be interesting to know the nature of the background endometrium of the L1CAM positive EECs in the multicenter study of Zeimet *et al*.[45]

Furthermore, up-regulation of L1CAM is associated with loss of E-cadherin, and loss of ER and PR expression.[47] Since L1CAM expression is a predictor of poor clinical survival, an association with other predictors of poor survival is not surprising.[14,31] A causal link between L1CAM and E-cadherin possibly lies within the Epithelial-Mesenchymal Transition (EMT) program. The EMT signaling pathway is identified to be the regulator of invasion and metastasis in cancer of epithelial origin. It is loosely defined by three major changes in cellular phenotype: (1) morphological changes from cobblestone-like epithelial cells to spindle-shaped mesenchymal cells which migrate more easily; (2) changes in markers of cell-cell junction such as E-cadherin; and (3) functional changes from stationary cells to motile cells.[39,48]

Recently, EMT was described in endometrial cancer as well.[49,50] These review articles can put the findings of Chapter 6, 7, and 8 in a broader perspective. The most common hallmarks of EMT have been found in endometrial carcinoma, either at the level of E-cadherin loss or at the induction of its repressors. Also other molecular alterations, consistent with the mesenchymal phenotype like L1CAM and MIB1 up-regulation, have been found in endometrial carcinoma. EMT inducer TGFβ1 was found to be one of the regulators of L1CAM expression, and MIB1 up-regulation has been found to contribute to the EMT-derived invasive phenotype in endometrial carcinoma cells.[49] Taking this all together, carcinomas evaluated in this thesis which were associated with poor clinical features like poor progression-free survival or metastatic disease, expressed features of the EMT pathway.

Conclusions

The endometrium of asymptomatic postmenopausal women shows focal proliferation in a substantial part of the patients, which possibly indicates that some EECs arise in focal areas of proliferative endometrium. Nevertheless, carcinomas in atrophic background endometrium and carcinomas in hyperplastic background endometrium show partly the

same IHC pattern and profile of mutations, but partly there are differences. This means that the carcinogenic process of EECs with atrophic background endometrium could be different from the known stepwise carcinogenic model of EEC.

Immunohistochemical expression of features known to be involved in carcinogenesis and metastases of EECs could be useful in daily clinical practice. It could be helpful in the discrimination between carcinomas with and without metastatic potential, and thus in identifying patients in need of more aggressive treatment.

Besides immunohistochemistry, conventional pathologic markers, such as cervical cytology, background endometrium, and depth of myometrial invasion could also be incorporated for prediction of the clinical course. This thesis gives new possibilities for the use of myometrial invasion and emphasizes the importance of looking at the tissue surrounding the tumor, since the nature of the background endometrium is a prognostic marker.

Future Perspectives

Before the results of this thesis can be translated into clinical use, data should be confirmed in a larger, prospective, multicentre trial. A set of IHC markers could be incorporated in an algorithm for pre-operative clinical decision making together with the already used pathologic features tumor type and tumor grade. This set of markers could consist of ER, PR, p16, p21, p53, E-cadherin, MIB1, and L1CAM, and depending on the results of future studies of more markers. At present, other researchers are investigating the prognostic use of IHC markers in endometrial carcinoma. Recently, the MOMaTeC study group proved that double negative hormone receptor status in pre-operative biopsy is an independent predictor of poor clinical outcome and lymph node metastases.[51] Other results of this multicenter, prospective trial are awaited. Currently, another prospective study investigates the usefulness of a set of IHC markers in the pre-operative setting of endometrial carcinoma patients (PIPENDO).[52]

Furthermore, a substantial part of the carcinogenesis of endometrial carcinoma is still not fully understood. Comparison of more IHC markers and mutations between EECs with atrophic and hyperplastic background endometrium should be performed in future research to prove the existence of a separate carcinogenic pathway. In addition, mutation analyses of background endometrium could give more information on the carcinogenesis of EECs.

The individualization of endometrial carcinoma treatment still needs fine tuning. Currently, we are at a point where we have gained more insight into endometrial carcinogenesis, but these insights are hardly used in daily clinical practice. More understanding of the process of tumor progression gives the possibility of using IHC or molecular markers for risk assessment for metastatic or recurrent disease, and gives potential for targeted therapy.

Suggestions for clinical practice

- In case of atypical or malignant endometrial cells in cervical cytology, the clinician should think about the possibility of a more aggressive endometrial carcinoma.
- Myometrial invasion should be measured in absolute millimeters of invasion instead of the currently used cut-off value of more or less than 50% of the myometrial thickness.
- The background endometrium should be mentioned in the pathology report of endometrial carcinoma patients.
- A set of IHC markers with ER, PR, MIB1, E-cadherin, and L1CAM should be incorporated in pre-operative and post-operative setting for guidance in clinical decision making.

References

1. McCluggage WG. Benign Diseases of the Endometrium. In: Kurman RJ, Ellenson LH, Ronnett BM, editors. Blaustein's Pathology of the Female Genital Tract. 6th ed: Springer; 2011. p. 305-58.
2. Buckley CHF, H. Biopsy pathology of the endometrium. London: Arnold; 2002.
3. Prior JC, Hitchcock CL. The endocrinology of perimenopause: need for a paradigm shift. Frontiers in bioscience (Scholar edition). 2011;3:474-86. Epub 2011/01/05.
4. Prior JC. Ovarian aging and the perimenopausal transition: the paradox of endogenous ovarian hyperstimulation. Endocrine. 2005;26(3):297-300. Epub 2005/07/22.
5. Sivridis E, Giatromanolaki A. Proliferative activity in postmenopausal endometrium: the lurking potential for giving rise to an endometrial adenocarcinoma. J Clin Pathol. 2004;57(8):840-4. Epub 2004/07/29.
6. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol. 1983;15(1):10-7. Epub 1983/02/01.
7. Ellenson HL, Ronnett BM, Kurman RJ. Precursor lesions of endometrial carcinoma. In: Kurman RJ, Ellenson HL, Ronnett BM, editors. Blaustein's Pathology of the Female Genital Tract. Sixth ed. New York: Springer; 2011. p. 359-91.
8. Ellenson HL, Ronnett BM, Soslow RA, Zaino RJ, Kurman RJ. Endometrial carcinoma. In: Kurman RJ, Ronnett BM, Ellenson HL, editors. Blaustein's Pathology of the Female Genital Tract. Sixth ed. New York: Springer; 2011. p. 393-452.
9. Sasaki H, Nishii H, Takahashi H, Tada A, Furusato M, Terashima Y, et al. Mutation of the Ki-ras protooncogene in human endometrial hyperplasia and carcinoma. Cancer Res. 1993;53(8):1906-10. Epub 1993/04/15.
10. Enomoto T, Inoue M, Perantoni AO, Buzard GS, Miki H, Tanizawa O, et al. K-ras activation in premalignant and malignant epithelial lesions of the human uterus. Cancer Res. 1991;51(19):5308-14. Epub 1991/10/01.
11. Guerrero S, Casanova I, Farre L, Mazo A, Capella G, Mangues R. K-ras codon 12 mutation induces higher level of resistance to apoptosis and predisposition to anchorage-independent growth than codon 13 mutation or proto-oncogene overexpression. Cancer Res. 2000;60(23):6750-6. Epub 2000/12/16.
12. Singh A, Greninger P, Rhodes D, Koopman L, Violette S, Bardeesy N, et al. A gene expression signature associated with "K-Ras addiction" reveals regulators of EMT and tumor cell survival. Cancer cell. 2009;15(6):489-500. Epub 2009/05/30.
13. Takeichi M. Cadherin cell adhesion receptors as a morphogenetic regulator. Science. 1991;251(5000):1451-5. Epub 1991/03/22.
14. Moreno-Bueno G, Hardisson D, Sarrio D, Sanchez C, Cassia R, Prat J, et al. Abnormalities of E- and P-cadherin and catenin (beta-, gamma-catenin, and p120ctn) expression in endometrial cancer and endometrial atypical hyperplasia. J Pathol. 2003;199(4):471-8. Epub 2003/03/14.
15. Pijnenborg JM, Kisters N, van Engeland M, Dunselman GA, de Haan J, de Goeij AF, et al. APC, beta-catenin, and E-cadherin and the development of recurrent endometrial carcinoma. Int J Gynecol Cancer. 2004;14(5):947-56. Epub 2004/09/14.

16. Sivridis E, Fox H, Buckley CH. Endometrial carcinoma: two or three entities? Int J Gynecol Cancer 1998;8:183-8.
17. Geels YP, Pijnenborg JM, van den Berg-van Erp SH, Bulten J, Visscher DW, Dowdy SC, et al. Endometrioid endometrial carcinoma with atrophic endometrium and poor prognosis. Obstet Gynecol. 2012;120(5):1124-31. Epub 2012/10/24.
18. Creutzberg CL, van Putten WL, Koper PC, Lybeert ML, Jobsen JJ, Warlam-Rodenhuis CC, et al. Surgery and postoperative radiotherapy versus surgery alone for patients with stage-1 endometrial carcinoma: multicentre randomised trial. PORTEC Study Group. Post Operative Radiation Therapy in Endometrial Carcinoma. Lancet. 2000;355(9213):1404-11. Epub 2000/05/03.
19. Todo Y, Minobe S, Okamoto K, Takeda M, Ebina Y, Watari H, et al. Cytological features of cervical smears in serous adenocarcinoma of the endometrium. Jpn J Clin Oncol. 2003;33(12):636-41. Epub 2004/02/11.
20. Skaznik-Wikiel ME, Ueda SM, Frasure HE, Rose PG, Fleury A, Grumbine FC, et al. Abnormal cervical cytology in the diagnosis of uterine papillary serous carcinoma: earlier detection of a poor prognostic cancer subtype? Acta Cytol. 2011;55(3):255-60. Epub 2011/04/29.
21. Fukuda K, Mori M, Uchiyama M, Iwai K, Iwasaka T, Sugimori H, et al. Preoperative cervical cytology in endometrial carcinoma and its clinicopathologic relevance. Gynecol Oncol. 1999;72(3):273-7. Epub 1999/03/04.
22. Kinde I, Bettegowda C, Wang Y, Wu J, Agrawal N, Shih Ie M, et al. Evaluation of DNA from the Papanicolaou test to detect ovarian and endometrial cancers. Science translational medicine. 2013;5(167):167ra4. Epub 2013/01/11.
23. Zaino RJ, Kurman RJ, Diana KL, Morrow CP. Pathologic models to predict outcome for women with endometrial adenocarcinoma: the importance of the distinction between surgical stage and clinical stage--a Gynecologic Oncology Group study. Cancer. 1996;77(6):1115-21. Epub 1996/03/15.
24. Larson DM, Connor GP, Broste SK, Krawisz BR, Johnson KK. Prognostic significance of gross myometrial invasion with endometrial cancer. Obstet Gynecol. 1996;88(3):394-8. Epub 1996/09/01.
25. Kondalsamy-Chennakesavan S, van Vugt S, Sanday K, Nicklin J, Land R, Perrin L, et al. Evaluation of tumor-free distance and depth of myometrial invasion as prognostic factors for lymph node metastases in endometrial cancer. Int J Gynecol Cancer. 2010;20(7):1217-21. Epub 2011/04/19.
26. Chattopadhyay S, Galaal KA, Patel A, Fisher A, Nayar A, Cross P, et al. Tumour-free distance from serosa is a better prognostic indicator than depth of invasion and percentage myometrial invasion in endometrioid endometrial cancer. BJOG : an international journal of obstetrics and gynaecology. 2012;119(10):1162-70. Epub 2012/07/19.
27. Lindauer J, Fowler JM, Manolitsas TP, Copeland LJ, Eaton LA, Ramirez NC, et al. Is there a prognostic difference between depth of myometrial invasion and the tumor-free distance from the uterine serosa in endometrial cancer? Gynecol Oncol. 2003;91(3):547-51. Epub 2003/12/17.
28. Kaku T, Tsuruchi N, Tsukamoto N, Hirakawa T, Kamura T, Nakano H. Reassessment of myometrial invasion in endometrial carcinoma. Obstet Gynecol. 1994;84(6):979-82. Epub 1994/12/01.

29. Vandenput I, Trovik J, Leunen K, Wik E, Stefansson I, Akslen L, et al. Evolution in endometrial cancer: evidence from an immunohistochemical study. Int J Gynecol Cancer. 2011;21(2):316-22. Epub 2011/07/08.
30. Suthipintawong C, Wejaranayang C, Vipupinyo C. Prognostic significance of ER, PR, Ki67, c-erbB-2, and p53 in endometrial carcinoma. J Med Assoc Thai. 2008;91(12):1779-84. Epub 2009/01/13.
31. Oreskovic S, Babic D, Kalafatic D, Barisic D, Beketic-Oreskovic L. A significance of immunohistochemical determination of steroid receptors, cell proliferation factor Ki-67 and protein p53 in endometrial carcinoma. Gynecol Oncol. 2004;93(1):34-40. Epub 2004/03/30.
32. Huvila J, Talve L, Carpen O, Edqvist PH, Ponten F, Grenman S, et al. Progesterone receptor negativity is an independent risk factor for relapse in patients with early stage endometrioid endometrial adenocarcinoma. Gynecol Oncol. 2013;130(3):463-9. Epub 2013/06/20.
33. Campisi J, d'Adda di Fagagna F. Cellular senescence: when bad things happen to good cells. Nature reviews Molecular cell biology. 2007;8(9):729-40. Epub 2007/08/02.
34. Ignatov A, Bischoff J, Schwarzenau C, Krebs T, Kuester D, Herrmann K, et al. P16 alterations increase the metastatic potential of endometrial carcinoma. Gynecol Oncol. 2008;111(2):365-71. Epub 2008/09/16.
35. Salvesen HB, Das S, Akslen LA. Loss of nuclear p16 protein expression is not associated with promoter methylation but defines a subgroup of aggressive endometrial carcinomas with poor prognosis. Clin Cancer Res. 2000;6(1):153-9. Epub 2000/02/03.
36. Stewart CJ, Crook ML, Leung YC, Platten M. Expression of cell cycle regulatory proteins in endometrial adenocarcinoma: variations in conventional tumor areas and in microcystic, elongated and fragmented glands. Mod Pathol. 2009;22(5):725-33. Epub 2009/03/10.
37. Hecht JL, Mutter GL. Molecular and pathologic aspects of endometrial carcinogenesis. J Clin Oncol. 2006;24(29):4783-91. Epub 2006/10/10.
38. Jacks T, Weinberg RA. Cell-cycle control and its watchman. Nature. 1996;381(6584):643-4. Epub 1996/06/20.
39. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74. Epub 2011/03/08.
40. Salvesen HB, Iversen OE, Akslen LA. Prognostic significance of angiogenesis and Ki-67, p53, and p21 expression: a population-based endometrial carcinoma study. J Clin Oncol. 1999;17(5):1382-90. Epub 1999/05/20.
41. Steinbakk A, Malpica A, Slewa A, Skaland I, Gudlaugsson E, Janssen EA, et al. Biomarkers and microsatellite instability analysis of curettings can predict the behavior of FIGO stage I endometrial endometrioid adenocarcinoma. Mod Pathol. 2011;24(9):1262-71. Epub 2011/05/10.
42. Saegusa M, Hashimura M, Suzuki E, Yoshida T, Kuwata T. Transcriptional up-regulation of Sox9 by NF-kappaB in endometrial carcinoma cells, modulating cell proliferation through alteration in the p14(ARF)/p53/p21(WAF1) pathway. The American journal of pathology. 2012;181(2):684-92. Epub 2012/06/16.
43. Horree N, van Diest PJ, van der Groep P, Sie-Go DM, Heintz AP. Progressive derailment of cell cycle regulators in endometrial carcinogenesis. J Clin Pathol. 2008;61(1):36-42. Epub 2007/05/08.

44. Fogel M, Huszar M, Altevogt P, Ben-Arie A. L1 (CD171) as a novel biomarker for ovarian and endometrial carcinomas. Expert Rev Mol Diagn. 2004;4(4):455-62. Epub 2004/07/01.
45. Zeimet AG, Reimer D, Huszar M, Winterhoff B, Puistola U, Abdel Azim S, et al. L1CAM in Early-Stage Type I Endometrial Cancer: Results of a Large Multicenter Evaluation. J Natl Cancer Inst. 2013. Epub 2013/06/20.
46. Fogel M, Gutwein P, Mechtersheimer S, Riedle S, Stoeck A, Smirnov A, et al. L1 expression as a predictor of progression and survival in patients with uterine and ovarian carcinomas. Lancet. 2003;362(9387):869-75. Epub 2003/09/19.
47. Huszar M, Pfeifer M, Schirmer U, Kiefel H, Konecny GE, Ben-Arie A, et al. Up-regulation of L1CAM is linked to loss of hormone receptors and E-cadherin in aggressive subtypes of endometrial carcinomas. J Pathol. 2010;220(5):551-61. Epub 2010/01/16.
48. Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Developmental cell. 2008;14(6):818-29. Epub 2008/06/10.
49. Colas E, Pedrola N, Devis L, Ertekin T, Campoy I, Martinez E, et al. The EMT signaling pathways in endometrial carcinoma. Clinical & translational oncology : official publication of the Federation of Spanish Oncology Societies and of the National Cancer Institute of Mexico. 2012;14(10):715-20. Epub 2012/08/23.
50. Mirantes C, Espinosa I, Ferrer I, Dolcet X, Prat J, Matias-Guiu X. Epithelial-to-mesenchymal transition and stem cells in endometrial cancer. Hum Pathol. 2013;44(10):1973-81. Epub 2013/07/13.
51. Trovik J, Wik E, Werner HM, Krakstad C, Helland H, Vandenput I, et al. Hormone receptor loss in endometrial carcinoma curettage predicts lymph node metastasis and poor outcome in prospective multicentre trial. European journal of cancer (Oxford, England : 1990). 2013;49(16):3431-41. Epub 2013/08/13.
52. http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=3503. 2012.

SUMMARY

In the western world, endometrial cancer is the most common cancer of the female genital tract. In a generally accepted dualistic model of endometrial carcinogenesis two subtypes are distinguished based on clinical behavior and histopathologic presentation of the carcinoma. Type I carcinoma, representing 80% of all endometrial carcinomas, occurs around an average age of 60 years and bears in general a good prognosis. These endometrioid carcinomas are directly linked to unopposed estrogen stimulation, and show a background of hyperplastic endometrium. Type I carcinomas are characterized by ER and PR expression, Micro Satellite Instability, and alterations in *KRAS, CTNNB1, PTEN,* and the WNT-pathway. Type II carcinomas, on the contrary, occur at an average age of 70 years, and bear a relatively poor prognosis. These carcinomas show non-endometrioid histology like serous and clear cell type. The background endometrium of these patients appears atrophic, and carcinomas are characterized by aneuploidy, p53 and Her2/neu expression, and loss of E-cadherin expression. An outline of the treatment strategies and the currently known predictive markers for endometrial carcinoma is given in **Chapter 1**.

In order to investigate endometrial carcinogenesis, the physiological conditions of the (postmenopausal) endometrium should be clear. **Chapter 2** gives a detailed description of the endometrium of 20 premenopausal and 48 postmenopausal women who did not have symptoms of (abnormal) vaginal bleeding. To this end, the endometrium of these patients undergoing a hysterectomy for symptoms of uterine prolapse was analyzed systematically using the SEE-END protocol. (Focal) proliferative endometrium or hyperplasia was found in 33% of the postmenopausal women. Hyperplasia or disordered proliferative endometrium was found in 5% of the premenopausal women. This is a remarkable high prevalence of endometrial pathology in asymptomatic women, but the clinical relevance of these lesions has yet to be determined.

In **Chapter 3**, we found abnormal endometrial cells in cervical cytology in 87.5% of a cohort 80 uterine papillary serous carcinoama (UPSC) patients, and in 37.8% of a cohort of 267 endometrioid endometrial carcinoma (EEC) patients. Furthermore, abnormal endometrial cells in cervical cytology were associated with extra-uterine spread of disease in the UPSC group and with cervical involvement in the EEC group. These data indicate that the clinician

should be more alert for a non-endometrioid carcinoma or an endometrioid carcinoma in advanced stage when endometrial cells are found in cervical cytology.

Currently, myometrial invasion is expressed as more or less than 50% of the total myometrium. In literature, the interobserver variability of assessing myometrial invasion is around 30%. In **Chapter 4** the predictive value of expressing myometrial invasion as the absolute depth of invasion in millimeters was compared to expressing myometrial invasion in more or less than 50% of the myometrium, and to expressing invasion as the distance to the serosa in millimeters in a cohort of 335 EEC patients. The absolute depth of myometrial invasion was found to have a better predictive value than the other ways of measuring myometrial invasion. Whether the interobserver variability decreases with this way of measuring myometrial invasion has yet to be determined.

Approximately 20% of the individual cases does not fit the dualistic model of endometrial carcinogenesis. This 20% includes endometrioid carcinomas associated with poor prognosis and atrophic background endometrium. In **Chapter 5** the background endometrium of a cohort of 527 tumor grade 1 endometrioid endometrial carcinomas was analyzed. Seventeen percent showed atrophic background endometrium. In addition, atrophic background endometrium was an independent prognostic factor for patients with tumor grade 1 EEC. Possibly, these carcinomas with atrophic background endometrium do not follow the hypothesized progression model for type I tumors. They may arise trough a unique, third carcinogenic pathway.

With the intention to find more evidence for the existence of a third type endometrial carcinoma in **Chapter 6**, we compared the expression profile of a set immunohistochemical (IHC) markers, and the presence of *BRAF, KRAS,* and *PIK3CA* mutations in EEC patients with hyperplastic background endometrium (type I), with UPSC patients (type II), and EEC patients with atrophic background endometrium (type III). Expected differences between EEC (type I and III) and UPSC (type II) were found: EEC showed higher expression of ER and PR, and lower expression of L1CAM, p53, and MLH1. Within the EECs, the expression patterns of type I and type III carcinomas were mostly comparable. However, type III carcinomas did show significantly lower E-cadherin expression, and significantly less mutations in the *KRAS* gene than type I carcinomas. *KRAS* mutation comprises an important step in the type I carcinogenic model of hyperplasia turning into EEC. These findings might indicate that type III carcinomas lack a crucial step in the carcinogenic pathway of type I carcinomas.

In **Chapter 7**, in order to identify prognostic markers for EEC, a comparison of the expression profile of a set of 12 IHC markers was made between the primary tumor of EEC patients with metastatic disease and the primary tumor of a control group of EEC patients with FIGO stage I disease. Only ER expression was found to be significantly lower in patients with metastatic disease. This finding emphasizes that loss of ER expression is an important predictor of poor clinical outcome. A second evaluation in this chapter showed significantly more p16 and p21 expression in the metastatic site when compared to the primary tumor. this indicates that these two effectors of the Rb and P53 pathway respectively, play a role in the metastatic process. The third comparison showed increased MIB1 expression and decreased ER and PR expression in the primary tumor of patients with distant metastases when compared to the primary tumor of patients with loco-regional metastases.

In **Chapter 8**, L1CAM was studied to determine if it was associated with clinicopathologic features in EEC. L1CAM, a membranous marker, regulating cell-cell interactions, has recently been reported by other authors to be an independent predictor of survival in EEC. In our study, 18 of a cohort of 103 EEC patients was L1CAM positive. However, after histologic review, 11 of the 18 L1CAM positive cases turned out to be non-endometrioid carcinomas. L1CAM was associated with older age, poor tumor differentiation, lymphovascular space invasion, and was a predictor of poor survival in univariable analysis. In multivariable analysis non-endometrioid histology, and advanced FIGO stage turned out to be independent predictors of survival, as L1CAM was not. We concluded from this study that L1CAM expression could support correct classification of endometrial carcinoma and that it carries prognostic information for endometrial carcinoma.

In **Chapter 9** the results of the different chapters are discussed and put in perspective with results from literature. In addition, some clinical recommendations are done, and the possibilities for future research are discussed.

SAMENVATTING

In de westerse wereld is het endometriumcarcinoom de meest voorkomende maligniteit van de vrouwelijke tractus genitalis. In een algemeen geaccepteerd, dualistisch model voor de carcinogenese van het endometriumcarcinoom worden twee typen onderscheiden. Dit onderscheid is gebaseerd op het klinische gedrag en de histopathologische presentatie van het carcinoom. Type I carcinomen, goed voor 80% van alle endometriumcarcinomen, ontstaan rond een gemiddelde leeftijd van 60 jaar en over het algemeen hebben deze patiënten een goede prognose. Deze endometrioïde carcinomen zijn het directe gevolg van stimulatie door oestrogenen en ontstaan in een achtergrond van hyperplastisch endometriumweefsel. Type I carcinomen worden gekarakteriseerd door expressie van ER en PR, Microsatelliet instabiliteit en veranderingen in *KRAS, CTNNB1, PTEN* en de WNT-signaaltransductiecascade. Dit in tegenstelling tot de type II carcinomen, die rond een gemiddelde leeftijd van 70 jaar ontstaan en over het algemeen een slechte prognose hebben. Type II carcinomen laten vaak het histologische beeld zien van een niet-endometrioïd type carcinoom zoals het sereuze carcinoom of het helder-cellige carcinoom. Het achtergrondendometrium van deze patiënten is atrofisch. Deze carcinomen worden gekenmerkt door aneuploïdie, p53 en Her2/neu expressie en verlies van E-cadherine expressie. **Hoofdstuk 1** geeft een overzicht van de behandelmethodes en de voorspellende markers voor het endometriumcarcinoom die we op dit moment kennen.

Om meer inzicht te krijgen in de carcinogenese van het endometriumcarcinoom, zouden we meer moeten weten over de fysiologie van het (postmenopauzale) endometrium. **Hoofdstuk 2** geeft een gedetailleerde beschrijving van het endometrium van 20 premenopauzale en 48 postmenopauzale vrouwen die geen symptomen hadden van (abnormaal) vaginaal bloedverlies. Om dit te bewerkstelligen werd het endometrium van deze vrouwen, die een hysterectomie ondergingen vanwege een prolaps uteri, systematisch onderzocht volgens het SEE-END protocol. (Focaal) proliferatief endometrium of hyperplastisch endometrium werd gevonden bij 33% van de postmenopauzale vrouwen. Hyperplastisch endometrium, of abnormaal proliferatief endometrium werd gevonden bij 5% van de premenopauzale vrouwen. Dit is een opmerkelijk hoge prevalentie van pathologie van het endometrium van vrouwen zonder symptomen van (abnormaal) vaginaal bloedverlies. De klinische betekenis van deze bevindingen is echter nog niet geheel duidelijk.

In **hoofdstuk 3** vonden we abnormale endometriumcellen in het cervixuitstrijkje in 87.5% van een cohort van 80 patiënten met sereuze carcinomen en in 37.8% van een cohort van 267 patiënten met endometrioïde endometrium carcinomen (EEC). Bovendien waren abnormale endometriumcellen in het cervixuitstrijkje geassocieerd met uitbreiding van het carcinoom buiten de uterus van de groep patiënten met sereuze tumoren en met uitbreiding van het carcinoom in de cervix van de groep patiënten met endometrioïde tumoren. Deze data geven een indicatie dat de clinicus gewaarschuwd moet zijn voor de mogelijkheid van ofwel een niet-endometrioïd carcinoom ofwel een endometrioïd carcinoom in een hoog stadium wanneer endometriumcellen worden gevonden in het cervixuitstrijkje.

Volgens de huidige richtlijnen wordt invasie van de tumor in het myometrium uitgedrukt in meer of minder dan 50% van de totale dikte van het myometrium. Uit studies blijkt dat in ongeveer 30% van de gevallen de pathologen die de diepte van de myometriuminvasie beoordelen van mening verschillen. In **hoofdstuk 4** wordt de voorspellende waarde van het uitdrukken van myometriuminvasie als absoluut getal in millimeters vergeleken met het uitdrukken van myometriuminvasie in meer of minder dan 50% en met het uitdrukken van de myometriuminvasie in millimeters naar de serosa in een cohort van 335 EEC patiënten. De myometriuminvasie uitgedrukt als absoluut getal in millimeters had een betere voorspellende waarde dan de andere manieren van meten en uitdrukken van myometriuminvasie. Het moet nog worden uitgezocht of de beoordelingen van verschillende pathologen beter met elkaar overeen zullen komen door deze andere manier van meten te gebruiken.

Ongeveer 20% van de individuele casus past niet in het dualistische model van de carcinogenese van het endometriumcarcinoom. Deze 20% behelst endometrioïde carcinomen die geassocieerd zijn met een slechte prognose en atrofisch achtergrondendometrium. In **hoofdstuk 5** werd het achtergrondendometrium van een cohort van 527 patiënten met graad 1 endometrioïde endometriumcarcinomen (EEC) geanalyseerd. Zeventien procent had atrofisch achtergrondendometrium. Daarnaast was atrofisch achtergrondendometrium een onafhankelijke voorspeller van de klinische uitkomst van patiënten met graad 1 endometrioïd endometriumcarcinoom. Het is mogelijk dat deze carcinomen met atrofisch achtergrondendometrium niet het stapsgewijze model volgen voor de carcinogenese van het type I endometriumcarcinoom. Wellicht ontstaan deze carcinomen door een ander, derde model voor carcinogenese.

Met de bedoeling om meer bewijs te vinden voor dit mogelijke derde type endometriumcarcinoom, hebben we in **hoofdstuk 6** het expressiepatroon van een set van immunohistochemische (IHC) markers en de aanwezigheid van *BRAF, KRAS* en *PIK3CA* mutaties bij EEC patiënten met hyperplastisch endometrium (type I), patiënten met sereuze carcinomen (type II) en EEC patiënten met atrofisch endometrium (type III) vergeleken. We vonden de verwachte verschillen tussen EEC (type I en III) en sereuze carcinomen (type II): EEC liet een hogere expressie van ER en PR zien en een lagere expressie van L1CAM, p53 en MLH1. Bij de vergelijking tussen de EEC patiënten, waren de expressiepatronen van type I en III voor het grootste deel vergelijkbaar. Type III liet wel een significant lagere E-cadherine expressie zien. Daarnaast waren er significant minder mutaties van het *KRAS* gen dan bij het type I carcinoom. *KRAS* mutaties zijn een belangrijke stap in het model voor het ontstaan van type I carcinomen bij de overgang van hyperplastisch endometrium naar graad I EEC. Deze bevindingen geven een aanwijzing dat type III carcinomen een cruciale stap overslaan in het model voor carcinogenese van type I carcinomen.

In **hoofdstuk 7** hebben we het expressie profiel van een set van 12 IHC markers in de primaire tumor van een groep patiënten met FIGO stadium I EEC vergeleken met het expressiepatroon in de primaire tumor van een groep EEC patiënten met metastasen. Het doel van deze vergelijking was het identificeren van prognostische markers voor EEC patiënten. Alleen ER expressie was significant hoger in de groep met metastasen. Deze bevinding benadrukt dat verlies ER expressie een belangrijke voorspeller is van een slechte klinische uitkomst. Een tweede vergelijking in dit hoofdstuk liet significant meer expressie van p16 en p21 zien in de metastase vergeleken met de primaire tumor van dezelfde patiënt. Dit kan betekenen dat deze twee effectoren van de, respectievelijk, Rb en P53 signaaltransductiecascade een rol spelen bij het metastaseringsproces. De derde vergelijking liet verhoogde expressie van MIB1 en verlaagde expressie van ER en PR zien in de primaire tumor van patiënten met metastasen op afstand, vergeleken met de primaire tumor van patiënten met lokale of regionale metastasen.

In **hoofdstuk 8** werd L1CAM bestudeerd om te bepalen of deze marker geassocieerd is met klinische en pathologische uitkomsten bij EEC patiënten. L1CAM, een membraneuze marker, reguleert de cel- cel interactie. Het is recent aangetoond door andere auteurs dat het een onafhankelijke voorspeller van overleving is voor EEC patiënten. Wij vonden dat bij 18 van een cohort van 103 EEC patiënten de tumor L1CAM positief was. Echter, na revisie van de

histologische coupes bleken 11 van de 18 L1CAM positieve tumoren niet-endometrioïde carcinomen te zijn. L1CAM was geassocieerd met oudere leeftijd, slechte tumordifferentiatie en invasie van de lymfovasculaire ruimtes en was verder geassocieerd met slechte overleving na univariate analyse. Bij multivariate analyse bleken niet-endometrioïde histologie en gevorderd FIGO stadium de onafhankelijke voorspellers van overleving, L1CAM was geen onafhankelijke voorspeller. Wij concludeerden uit deze studie dat L1CAM expressie ondersteunend kan zijn bij het stellen van de goede histologische diagnose en dat L1CAM prognostische informatie kan geven over het endometriumcarcinoom.

In **hoofdstuk 9** worden de resultaten van de verschillende hoofdstukken bediscussieerd en in perspectief geplaatst ten opzichte van eerdere resultaten uit de literatuur. Aanvullend worden een aantal klinische aanbevelingen gedaan en de mogelijkheden voor toekomstig onderzoek worden besproken.

DANKWOORD

Eindelijk mag ik dan het dankwoord van mijn proefschrift schrijven. Dit proefschrift was er natuurlijk niet geweest zonder de hulp en steun van een aantal mensen die ik hieronder wil noemen.

Om te beginnen mijn promotor, prof. dr. Massuger.

Beste Leon, jij hebt dit alles mogelijk gemaakt. Je bent een onuitputtelijke bron van ideeën en een meester in het vinden van mogelijkheden. Op de vele momenten dat je met ideeën kwam, heb ik regelmatig gedacht: “ik moet nog maar eens zien of dat gaat gebeuren” En ja, het is allemaal gebeurd! De parttime start, de fulltime baan, alle onderzoeken die we hebben opgezet, de samenwerking met buitenlandse klinieken, allemaal gelukt. Je liet mij inzien dat ik het zelf kon laten gebeuren. Dat is door de jaren heen een hele goede motivatie voor mij geweest.

Vervolgens mijn drie copromotoren: dr. Pijnenborg, dr. Bulten en dr. Snijders.

Beste Hanny, ontzettend bedankt voor het feit dat je er altijd was. Ieder moment dat ik feedback of een sparpartner nodig had, was jij er met een snelle reactie of tijd voor een telefoontje. De ideeën die je had waren altijd vernieuwend. Je bent iemand met een onuitputtelijke hoeveelheid energie en je enthousiasme werkt aanstekelijk. Met jouw input op de “endometriumlijn” weet ik zeker dat daar nog heel veel mooie onderzoeken uit gaan volgen.

Beste Hans, we hebben heel wat uurtjes samen achter de microscoop doorgebracht. Misschien heb ik je iets te vaak gevraagd wanneer je weer tijd had voor de onmogelijke hoeveelheid coupes die we moesten bestuderen. Veel dank voor je doorzettingsvermogen daarbij. Ik heb veel van je geleerd en heb ontzettend veel respect gekregen voor het moeilijke en uitdagende vak pathologie. Daarbij ook dank voor onze gesprekken en de toevoeging die je hebt gegeven aan mijn culturele opvoeding.

Beste Marc, in het CWZ begon het allemaal. Dank voor je begeleiding toen ik startte als arts-assistent gynaecologie en heel veel dank voor de mogelijkheid die je mij hebt gegeven door mij tijdens mijn baan als arts-assistent een dag in de week de tijd te geven om aan mijn onderzoek te werken. Zonder deze dag was de start van mijn promotietraject onmogelijk geweest. Daarnaast veel dank voor je kritische houding en correcties op mijn teksten. Het

kon even wat werk zijn als ik jouw correcties moest verwerken, maar dan had ik ook een goede tekst.

Dan heb ik nog een kleine toevoeging aan de dankbetuiging voor mijn “promotieteam”: de jaren van mijn promotietraject zijn door persoonlijke gebeurtenissen niet altijd even makkelijk geweest. Ik wil jullie alle vier ontzettend bedanken voor jullie steun en begrip!

Mijn dank gaat tevens uit naar de gehele maatschap gynaecologie van het Canisius-Wilhelmina Ziekenhuis voor het mogelijk maken van de start van mijn promotietraject door mij in staat te stellen een dag in de week aan mijn onderzoek te besteden.

Halverwege mijn promotietraject werd Dr. van Tilborg als postdoc aangesteld bij de afdeling pathologie en gynaecologie. Beste Angela, dank voor al jouw input bij het laatste deel van dit manuscript en het delen van jouw kennis over markers en mutatie-analyzes. Het was heel fijn om met je samen te werken.

Hulp van de pathologie kwam niet alleen uit het Radboudumc, maar ook uit het Canisius-Wilhelmina Ziekenhuis en het st. Elisabeth Ziekenhuis, van dr. van den Berg-van Erp en dr. van der Wurff. Beste Saskia en Anneke, veel dank voor het meedenken, mogelijk maken van materiaal verzamelen en reviseren van de coupes. Het was prettig om met jullie samen te werken.

Special thanks to Sean Dowdy and Daniel Visscher from the Mayo Clinic, Rochester, Minnesota, for your hospitality, and for giving me the opportunity to collaborate with you.

Bij de eerste studie waarmee ik van start ging, die uiteindelijk hoofdstuk 8 is geworden, mocht ik de database van dr. van Kempen gebruiken en aanvullen. Beste Léon, dank voor de samenwerking en voor de input die je ook vanuit Canada bent blijven geven. Er zullen nog vele studies volgen met de marker waarmee dit promotietraject begon.

Lieve (ex) collega’s van de tuin. Dank jullie wel voor de gezellige tijd! Dank voor de taart, voor het samen bestuderen van de vissen, voor de vrijdagmiddagborrels, de woensdag-breek-de-week-borrels, de zomerfeesten, de skiweekenden , de onderzoekersweekenden en niet te vergeten de fantastische congressen waar we samen zijn geweest! het leven van een promovendus is zo slecht nog niet… Marjanka, ik mis het nog steeds dat ik niet meer een computerscherm heb met iemand daarachter met wie ik altijd kan praten.

Louis, wat fijn dat jij mijn opvolger bent. Je hebt het staartje van mijn promotie een stuk eenvoudiger gemaakt. Veel succes met jouw promotietraject!

Birgit en Bart, dank voor al jullie hulp die jullie als student hebben geboden bij het verzamelen van de data.

Lieve nieuwe collega's van de huisartsopleiding, in het bijzonder mijn eerstejaars groepje. Wat een ontzettend leuke mensen zijn jullie. Ik heb me in onze groep vanaf het begin erg op mijn gemak gevoeld en een fantastisch eerste jaar gehad met jullie. Ik hoop jullie nog vaak te zien, vooralsnog gaat dat in ieder geval helemaal volgens plan.

Lieve Sabine en Dolores, jullie hebben me als opleiders enorm enthousiast gemaakt voor het huisartsen vak. Ik sta daardoor helemaal achter de keuze van mijn "nieuwe beroep". Daarnaast hebben jullie me af en toe net dat extra beetje ruimte gegeven om mijn promotie af te kunnen ronden. Dank daarvoor.

Zonder vrienden en familie ben ik nergens…

Lieve dames '01, het jaar dat we allemaal zijn begonnen met geneeskunde. We hebben elkaar tijdens onze studie stukje bij beetje steeds beter leren kennen en een fantastische tijd gehad. Hoe bestaat het dat je zoveel lieve en zorgzame vriendinnen kunt hebben? Saskia, Anne Els, Lenny, Lotte, Hilde en Hilde, wat jammer dat we nu een eindje uit elkaar wonen en niet meer zo makkelijk met elkaar af kunnen spreken (al lukt het ons eigenlijk nog best aardig). Desirée, wat fijn dat we lekker samen in Amsterdam wonen, we hebben nog heel wat cafés en restaurants om uit te testen. Marije, ik heb ontzettend genoten van onze gezamenlijke studie-, stap- en buitenland-carrière. Het is belachelijk dat we een paar jaar niet in dezelfde stad hebben gewoond. Ik heb je gemist en ben blij dat het nu weer is zoals het hoort.

Lieve reünisten van onafhankelijk medisch damesdispuut "Formosa Lasciva". Het leven met jullie is een feest. Onze studententijd was een feest, de zomerfeesten, carnaval, vakanties en zelfs treinreisjes waren een feest. Dank jullie wel voor alle mooie momenten. Fanny, Krista, Marijn, Marja, Eveline en Hanneke, hoe goed dat we samen weer op skivakantie gaan. Karlijn, ik hoop dat je volgend jaar weer mee gaat. En Marja, fijn dat we zoveel ritjes samen naar Nijmegen maken, dan wordt autorijden opeens quality time.

Lieve meiden uit Waalwijk. Wat fijn dat jullie er altijd waren en nog steeds zijn. Audrey, Eefje en Juliëtte, ik hoop dat we nog veel gesprekken over het leven gaan hebben, met een wijntje natuurlijk.

Lieve vrienden uit de vriendjes-app. Wat een fijne groep mensen om in terecht te komen als "nieuwe vriendin van". Zo voelt het inmiddels al lang niet meer, ik voel me helemaal thuis

bij jullie. Dank jullie wel daarvoor. De paasweekenden en feestjes waren supergezellig, dat er nog maar vele mogen volgen.

Mijn paranimfen, Hilde Luijks en Lotte van Dijk. Lieve Hilde, wat een geluk dat vanaf de vijfde klas onze planning bijna gelijk is gaan lopen. Je bent van alle markten thuis, vrolijk en uitgelaten als het kan, serieus en georganiseerd als het moet. Je staat altijd voor iedereen klaar. Ik heb zo vaak bij je aan mogen kloppen dat ik de tel al lang kwijt ben geraakt. Lieve Lotte, vanaf de eerste dag van de introductie van onze studententijd ben jij niet meer uit mijn leven weg te denken. Je hebt een heerlijke eigen kijk op dingen en lost alles op met je mooie lach. En niet onbelangrijk: jij bent er ook altijd. Meiden, dank jullie wel dat jullie me ook nu weer gaan bijstaan door mijn paranimf te zijn.

Natuurlijk mogen hier mijn ouders niet ontbreken. Lieve mama en papa, wat heb ik een mooi leventje dankzij jullie. Dankjewel dat jullie me altijd hebben gesteund, vertrouwen in mij hebben gehad en me vrij hebben gelaten in het maken van mijn keuzes. We hebben een moeilijke tijd achter de rug. Ik ben zo blij dat het allemaal goed is gekomen, wie had dat ooit durven hopen? Ook Hilde en Wouter wil ik graag bedanken. Gewoon, omdat jullie zijn wie jullie zijn: leukere broers en zussen bestaan niet. En Jessica, jij bent daar een goede toevoeging aan. Lieve Steef en Willy en de rest van mijn schoonfamilie. Dank jullie wel voor jullie warmte en steun.

Allerliefste Pim, bij jou heb ik alles gevonden wat ik me kan wensen. Het leven is zo fijn met jou!

CURRICULUM VITAE

Yvette Geels werd op 24 mei 1982 geboren te Kaatsheuvel (gemeente Loon Op Zand) in Noord- Brabant. Vlak voor haar middelbare schoolperiode verhuisde zij met haar ouders, zusje Hilde en broertje Wouter naar Waalwijk, waar zij naar het Dr. Mollercollege ging. In 2001 behaalde ze haar VWO diploma en had het geluk om direct daarna te mogen beginnen met de studie geneeskunde aan de Radboud Universiteit in Nijmegen.

Tijdens de studie geneeskunde heeft Yvette een jaar in het bestuur gezeten van de Medische Faculteitsvereniging Nijmegen. In de wachttijd naar haar coschappen volgde ze een keuze stage in Nicaragua over seksueel overdraagbare aandoeningen bij tieners. Tijdens het coschap gynaecologie in het Slingeland Ziekenhuis te Doetinchem werd haar interesse gewekt voor het vak gynaecologie. De coschappen werden afgesloten met het tropencoschap in Ghana, waar zij een onderzoek heeft uitgevoerd naar het inleiden van de bevalling bij intra-uterien overleden foetus. Het laatste onderdeel van de studie geneeskunde was de wetenschappelijke stage die zij op de afdeling gynaecologische oncologie mocht doen onder leiding van dr. Joanne de Hullu. Het onderwerp van deze stage was dubbeltumoren in zowel het ovarium als het endometrium.

In augustus 2008 behaalde zij haar arts-examen en begon als arts-assistent (ANIOS) gynaecologie in het Canisius-Wilhelmina Ziekenhuis te Nijmegen. Yvette ging door met het onderzoek van haar wetenschappelijke stage, wat nog niet helemaal was afgerond. Dit resulteerde in de vraag of ze de studie naar het endometriumcarcinoom voort wilde zetten en zij startte hierop met de onderzoeken die u in dit proefschrift kunt vinden. Na een jaar parttime aan het promotieonderzoek te hebben gewerkt, vervolgde ze het traject vanaf oktober 2010 als fulltime arts-onderzoeker bij de pijler gynaecologische oncologie, in samenwerking met de afdeling pathologie van het Radboudumc te Nijmegen (promotor prof. dr. Leon Massuger, co-promotoren dr. Hanny Pijnenborg, dr. Hans Bulten en dr. Marc Snijders). Twee van de onderzoeken in dit proefschrift waren in samenwerking met de Devision of Gynecologic Surgery van de Mayo Clinic, te Rochester, Minnesota, VS, waar Yvette een aantal maanden van haar promotietraject heeft doorgebracht.

Yvette is altijd erg enthousiast geweest over de gynaecologie en heeft met veel genoegen in zowel het Canisius-Wilhelmina Ziekenhuis als het Radboudumc gewerkt. Toch heeft zij gedurende haar promotietraject besloten het roer om te gooien. Sinds maart 2013 werkt zij

met veel plezier als huisarts in opleiding via de Vrije Universiteit Amsterdam. Yvette woont samen met Pim de Graaf in Amsterdam.

BIBLIOGRAPHY

The opinion of gynecologists on the management of early-stage, high-grade endometrioid endometrial cancer.
Geels YP, van der Putten LJ, van der Steen-Banasik EM, Snijders MP, Massuger LF, Pijnenborg JM.
Eur. J. Gynaec. Oncol. accepted for publication 2014 April.

High prevalence of atypical hyperplasia in the endometrium of epithelial ovarian cancer patients.
Mingels MJ, Masadah R, **Geels YP**, Otte-Höller I, de Kievit IM, van der Laak JA, van Ham MA, Bulten J, Massuger LF.
American Journal of Clinical Pathology. 2014 Aug.

Is pre-operative risk-assessment in laparoscopic treatment of presumed low-risk endometrial carcinoma effective?
van der Heijden PA, **Geels YP**, van den Berg-van Erp SH, Massuger LF, Snijders MP.
Gynecological Surgery. 2014 May;11(2):97-103.

L1CAM in early-stage type I endometrial cancer: results of a large multicentre evaluation.
Zeimet AG, Reimer D, Huszar M, Winterhoff B, Puistola U, Azim SA, Müller-Holzner E, Ben-Arie A, van Kempen LC, Petru E, Jahn S, **Geels YP**, Massuger LF, Amant F, Polterauer S, Lappi-Blanco E, Bulten J, Meuter A, Tanouye S, Oppelt P, Stroh-Weigert M, Reinthaller A, Mariani A, Hackl W, Netzer M, Schirmer U, Vergote I, Altevogt P, Marth C, Fogel M.
J Natl Cancer Inst. 2013 Aug;105(15):1142-50. Epub 2013 Jun 18.

Histopathologic assessment of the entire endometrium in asymptomatic women.
Mingels MJ, **Geels YP**, Pijnenborg JM, van der Wurff AA, van Tilborg AA, van Ham MA, Massuger LF, Bulten J.
Hum Pathol. 2013 Oct;44(10):2293-301. Epub 2013 Aug 9.

Cervical cytology in serous and endometrioid endometrial cancer.
Roelofsen T, **Geels YP**, Pijnenborg JM, van Ham MA, Zomer SF, van Tilburg JM, Snijders MP, Siebers AG, Bulten J, Massuger LF.
Int J Gynecol Pathol. 2013 Jul;32(4):390-8.

Absolute depth of myometrial invasion in endometrial cancer is superior to the currently used cut-off value of 50%.
Geels YP, Pijnenborg JM, van den Berg-van Erp SH, Snijders MP, Bulten J, Massuger LF.
Gynecol Oncol. 2013 May;129(2):285-91. Epub 2013 Feb 17.

Endometrioid endometrial carcinoma with atrophic endometrium and poor prognosis.
Geels YP, Pijnenborg JM, van den Berg-van Erp SH, Bulten J, Visscher DW, Dowdy SC, Massuger LF.
Obstet Gynecol. 2012 Nov;120(5):1124-31.

Why do women with double primary carcinoma of the endometrium and ovary have a favorable prognosis?
van Altena AM, **Geels YP**, Bulten J, Kiemeney LA, de Hullu JA, Massuger LF.
Int J Gynecol Pathol. 2012 Jul;31(4):344-51.

Prognostic factors in stage II/III/IV and stages III/IV endometrioid and serous adenocarcinoma of the endometrium. P. Mhawech-Fauceglia, R.F. Herrmann, J. Kesterson, I. Izevbaye, S. Lele, K. Odunsi. Eur J Surg Oncol 2010 Dec;36(12):1195-201.
Geels YP, Massuger LF, Pijnenborg JM.
Eur J Surg Oncol. 2011 Aug;37(8):734-5.

Comparing vaginal and sublingual administration of misoprostol for labour induction in women with intra-uterine fetal death.
Geels YP, de Gouberville MC, Visser L, van Asten HA.
Trop Doct. 2010 Apr;40(2):77-80.